Tyrosine and its Effect on Cognitive Function and Load-carriage Performance in the Heat

Nicole Coull

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By

Nicole Coull BSc (Hons)

A thesis submitted to the University of Bedfordshire in partial fulfilment of the requirements for the degree of Masters of Science by Research

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Author's Declaration

I declare that this thesis is entirely my own work. It is being submitted for the degree of MSc by Research at the University of Bedfordshire. It has not been submitted before for any degree or examination in any other University.

I declare that the word count of this thesis is 19,986 words in length from the introduction to the commencement of the bibliography.

Nicole Coull
2nd December 2015

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Published Work and Conference Presentations

Journal Papers

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Conferences Papers/Presentations

Taylor, L., Chrismas, B., Watson, P., Horsfall, R. & **Coull, N.** (2015). Pharmacokinetics of Acute Tyrosine Ingestion at Rest. European Congress of Sport Science (ECSS) – Annual Meeting, Malmo, Sweden (poster presentation).

Coull, N., Foster, J., Chrismas, B. & Taylor, L. (2015). Effect of Tyrosine Ingestion on Cognitive Function and Load Carriage Performance in the Heat. International Conference on Environmental Ergonomics (ICEE) – Portsmouth (oral presentation).

Coull, N., Watkins, S., Aldous, J., Warren, L., Chrismas, B., Dascombe, B., Mauger, A. & Taylor, L. (2014). Effect of Tyrosine Ingestion on Cognitive and Physical Performance Utilising an Intermittent Soccer Performance Test (iSPT) in a Warm Environment. BASES Student Conference – Portsmouth.

Aldous, J., Akubat, I., Chrismas, B., Watkins, S., **Coull, N**., Warren, L. & Taylor, L. (2013). Quantifying the Influence of a Hyper-thermic Environment on Soccer Specific Physiological and Performance Capacity Utilising a Validated Soccer Non-motorised Treadmill Protocol. European Congress of Sport Science (ECSS) – Barcelona.

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CHAPTER 2. Literature Review

CHAPTER 4: Experimental Chapter 1 – Pharmacokinetics of Acute Tyrosine Ingestion at Rest

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List of Abbreviations

°C	Degrees Celsius
5-HT	Serotonin
b min^{-1}	Beats per minute
BBB	Blood brain barrier
BCAA	Branched-chain amino acid
cm	Centimetres
CNS	Central nervous system
CON	Control
CV	Coefficient of variation
DA	Dopamine
EEG	Electroencephalography
ERP	Event related potentials
fMRI	Functional magnetic resonance imagine
h	Hours
HPLC	High performance liquid chromatography
HR	Heart rate
ICC	Interclass correction
Kg	Kilogram
km.h	Kilometres per hour
LNAA	Large neutral amino acid
mg	Milligrams
mg·kg body $mass^{-1}$	Milligrams per kilogram of body mass
min	Minutes
mL	Millilitres
mm	Millimetres
mmHg	Millimetres of mercury
ms	Milliseconds
n	Number
NA	Noradrenaline
PLA	Placebo
RH	Relative humidity
RPE	Rating of perceived exertion
RTIPE/RTIME	Readiness to invest physical/mental effort
SD	Standard deviation
TE	Typical error
T_{re}	Rectal temperature
T_{sk}	Skin temperature
TSS	Thermal sensation
TYR	Tyrosine
µmol/L	Micromoles per litre

Abstract

Prolonged exercise-heat-stress impairs both exercise performance and cognitive function. Military based operations are often performed in hot environmental conditions and thus performance and safety may be compromised which could be potentially life threatening. Acute ingestion of tyrosine (TYR), a catecholamine precursor, has been shown to improve aspects of cognitive function and mood during exposure to stressful environments, in military and sport specific settings. Currently, there is limited research exploring the optimal dose of TYR relative to blood values, to prescribe pre-exercise or before exposure to a stressor.

Therefore, the purpose of experimental chapter 1 was to investigate the effects of acute TYR ingestion strategies (0, 150 and 300 mg·kg body mass^{-1} TYR administered in 2 equal doses, 4 h apart) on serum TYR concentrations at rest. Twenty-one healthy males were randomly allocated to one of three groups (n = 7 per group); HIGH (300 mg·kg body mass^{-1} TYR in total), LOW (150 mg·kg body mass^{-1} TYR in total) and CON (sugar free squash placebo). Ingestion of TYR was double blinded and was administered in a drink form (dissolved in 250 mL sugar free squash) in two separate doses at both 0900 and 1300. Participants consumed a standardized breakfast (0800) and lunch (1200) prior to consumption of TYR and remained in the laboratory from 0900-1700 having blood drawn every hour from a cannula. Measures of gastric discomfort were also recorded. Significant differences in serum TYR concentrations were observed between groups ($p < 0.001$), with the HIGH dose (399 ± 69 μmol/L) resulting in the largest elevation compared to the LOW dose (279 ± 76 μmol/L) and CON (64 ± 11 μmol/L). Ingesting TYR as a double-dose did not significantly increase the peak in serum TYR compared to the first dose in both groups; LOW (221 vs 279 μmol/L) and HIGH dose (350 vs 399 μmol/L) ($p > 0.05$). No significant differences in gastric discomfort were observed between groups ($p > 0.05$). This study demonstrates that ingestion of a single dose of 150 mg·kg body mass^{-1} TYR may be sufficient to elevate serum TYR concentrations and that the peak in TYR concentration typically occurs 2 h post ingestion (without the need for a second identical dose 4 h later).

Experimental chapter 2 was designed to investigate the effects of TYR ingestion on steady state exercise, cognitive function and time-trial performance in the heat,

utilising the identified dose from the findings of experimental chapter 1. Eight recreationally active, healthy males visited the laboratory on four occasions (two familiarisation and two experimental conditions). In a double-blind, counter-balanced, crossover design participants ingested a placebo [PLA (250 mL sugar free squash)] or TYR (same as PLA plus 150 mg·kg body mass^{-1} TYR powder) 1 h pre-exercise. Participants completed a 60 min walk at 6.5 km.h, followed by a 2.4 km time-trial carrying a 25 kg backpack in 40°C; 30% RH. Cognitive function (vigilance, dual-task and simple reaction time) was assessed at 5 time-points; pre-ingestion, pre-exercise, 30 min into exercise, post 60 min exercise and post time-trial. Traditional physiological (HR), perceptual (RPE, TSS) and temperature (rectal and skin temperature) measures were recorded throughout exercise. A significant increase from pre-post exercise ($p < 0.01$) was observed for vigilance and dual-task FALSE scores, and for reaction time in both conditions. However, no significant difference was observed between TYR and PLA conditions in any of the cognitive tests measured ($p > 0.05$). Furthermore, no significant difference was observed in time-trial completion time ($F_{1,14} = 547.9$, $p = 0.74$) between TYR (19.78 ± 3.44 min) and PLA (20.29 ± 3.55 min). No significant differences were observed in any of the physiological, perceptual or temperature measures between conditions ($p > 0.05$).

The main findings presented within this thesis indicate that although it was identified that a single dose of 150 mg·kg body mass^{-1} was sufficient to significantly elevate serum TYR concentrations, ingestion of this dose did not influence cognitive function or time-trial performance in the heat. This is surprising since ingestion of similar doses have significantly improved aspects of cognitive function previously during exercise-heat-stress. In conclusion it appears that under the conditions of the present study, TYR is not a useful ergogenic aid. Future research should aim to elucidate the central effects of TYR to enable a better understanding of its mechanistic properties.

Key words: Heat-stress; central fatigue; tyrosine; cognitive function

CHAPTER 1: General Introduction

Exposure to extreme stressors can impede an individual's capability to perform both physical (González-Alonso *et al.*, 1999) and cognitive tasks (Banderet and Lieberman, 1989, Lane *et al.*, 2004). Strenuous activities undertaken within extreme environmental conditions, such as high heat and humidity, impose an additional strain on the body, resulting in an accelerated and premature onset of fatigue (Nybo *et al.*, 2014). Fatigue is a complex phenomenon, defined as the inability to sustain a given workload (Nybo and Nielsen, 2001b) and is suggested to occur at all levels of the brain-muscle pathway (Roelands and Meeusen, 2010). Originally, fatigue was primarily attributed to a 'metabolic endpoint' involving endogenous substrate depletion and various other peripheral mechanisms (Meeusen *et al.*, 2006b). However, it is now clear that there is also a significant involvement of the central nervous system (CNS), suggesting that both peripheral and central factors are implicated in fatigue development (Roelands and Meeusen, 2010, Nybo *et al.*, 2014).

Several theories exist to explain the notion of centrally mediated fatigue (Cheung and Sleivert, 2004), however the original 'central fatigue hypothesis' proposes that prolonged exercise and/or exposure to extreme stress alters the activity and synthesis of the central monoamines, serotonin (5-HT), dopamine (DA) and noradrenalin (NA) (Newsholme, 1987). An elevated ratio of brain DA:5-HT has been suggested to augment performance, whereas low ratios induce lethargy and losses in motivation (Davis and Bailey, 1997). Thus, DA and NA (converted from DA) are significant neurotransmitters involved in the execution of both physical and cognitive tasks due to their direct association with alterations in arousal, motivation and motor control (McMorris *et al.*, 2006, Watson, 2008). This knowledge proffers the opportunity to manipulate the CNS using nutritional intervention strategies to attenuate the onset of fatigue in stressful environments. This may be useful in an athletic, occupational and military setting, as small dietary mediated improvements can significantly influence performance in physical and cognitive tasks (Meeusen *et al.*, 2006a, Roelands and Meeusen, 2010, Baker, 2013, Meeusen, 2014).

The precursor for catecholamine synthesis is tyrosine (TYR), a non-essential amino acid found in protein rich dietary sources and synthesised in the liver from

phenylalanine (Wurtman *et al.*, 1980). An exogenous source of TYR through oral supplementation increases the ratio of TYR to other large neutral amino acids (LNAA) for competitive transportation across the blood-brain-barrier (BBB), resulting in a greater cerebral uptake and an increase in DA and NA synthesis (Glaeser *et al.*, 1979). Previous military research investigating the effects of TYR observed significant improvements in aspects of cognitive function during exposure to stressful environments such as cold (Banderet and Lieberman, 1989, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007) and hypoxia (Banderet and Lieberman, 1989), extended wakefulness (Neri *et al.*, 1995) and the physical/emotional stress nexus (Deijen *et al.*, 1999). More recently, research has focused on the effect of TYR in an athletic population, finding improvements in exercise capacity (Tumilty *et al.*, 2011) and cognitive function (Coull *et al.*, 2015) after TYR ingestion in a warm environment. However, further investigations have failed to replicate these findings, despite implementing similar exercise paradigms and dose administration strategies (Watson *et al.*, 2012, Tumilty *et al.*, 2014).

It appears that TYR ingestion has the potential to influence centrally mediated fatigue and possibly improve aspects of cognitive function, mood and exercise performance during acute stress (Meeusen, 2014). As many sporting events, military operations and occupational pursuits are performed in stressful environments, most commonly in hot conditions, the use of TYR may be beneficial. There is limited research examining the effects of TYR on performance (physical and cognitive) in a military/occupational based setting, specific to heat stress and thus further research is required in this area. Moreover, lack of data on the dose response of TYR restricts its use as a nutritional aid (Marriott and Thomas, 1994).

CHAPTER 2: Literature Review

2.1 Thermoregulation and Heat-stress

Humans are homoeothermic beings and constantly generate heat in all cells of the body as mechanical or thermal energy from metabolic activity (Cheung *et al.*, 2000, Gonzalez-Alonso, 2012). The human body is able to regulate this heat production by maintaining a thermal balance between heat gain and heat loss, to achieve a stable deep body temperature of ~37°C (Havenith, 1999). This balance is sufficient for the basic functions of the body at rest in normothermic conditions and is effortlessly maintained via heat loss mechanisms to dissipate excess heat (Havenith, 1999). The four main pathways (radiation ($\dot{R}$), conduction ($\dot{C}$), convection ($\dot{K}$) and evaporation ($\dot{E}$)) in which heat can be gained or lost can be defined in a simple heat balance equation, derived from the first law of Thermodynamics as follows:

$$\dot{S} = \dot{M} \pm \dot{W}_k \pm \dot{R} \pm \dot{C} \pm \dot{K} - \dot{E} \ (W{\cdot}m^{-2})$$

Where $\dot{S}$ represents heat storage, $\dot{M}$ represents metabolic heat production and $\dot{W}_k$ is the external work performed by the individual in question (Cheung, 2009).

During exercise, the production of heat and consequential rise in core body temperature is detected by the thermo-sensitive neurons in the preoptic-anterior hypothalamus, which receive afferent sensory input from thermoreceptors located in the skin and internal organs (Wendt *et al.*, 2007). These sensory signals are integrated within the hypothalamus, which allows for autonomic initiation of thermoregulatory responses appropriate for any given thermal stress (Wendt *et al.*, 2007, Nakamura, 2011). Typically, detection of increased body and brain temperature triggers a response whereby heat is transferred from the core to the skin down a temperature gradient and subsequently dissipated to the environment via cutaneous vasodilation and the above heat loss mechanisms (Cheung, 2009, Nybo *et al.*, 2014). However, when ambient temperature is higher than skin temperature, the body begins to gain heat (resulting in a positive value for $\dot{S}$ in the above equation) from the environment and therefore evaporation becomes the primary method of heat loss (Havenith, 1999).

Heat is lost as sweat evaporates from the skins surface, which subsequently cools the blood below, accounting for a large proportion of heat dissipation (Brotherhood, 2008). This mechanism is dependent upon the relative humidity (RH) of the environment as this alters the water vapour pressure gradient between the environment and the body. Thus if humidity is high, skin cooling via sweat vaporisation is reduced and as a consequence, heat production surpasses the capacity for heat loss, leading to an elevated body and brain temperature (Wendt *et al.*, 2007). Additionally, wearing clothing and protective garments will further compromise evaporative heat loss by impeding the natural movement of water vapour, consequently exacerbating the rate of heat gain to potentially dangerous levels (Havenith, 1999, Cheung, 2009).

2.2 Hyperthermia-induced Fatigue

A significant rise in body and brain temperature due to inadequate thermoregulation (hyperthermia) is associated with physiological and cellular dysfunction, heat exhaustion and if left untreated, may result in death (Hocking *et al.*, 2001, Cheung and Sleivert, 2004). It is difficult to distinguish between the effects of a high body temperature and that of a high brain temperature within the literature, as brain temperature is not easily measured during physical exercise. It has been suggested that brain temperature increases in parallel with that of core temperature during exercise. However, there is evidence to show that on average, the brain is slightly warmer (by 0.2-0.3°C) than the core due to its higher heat production and inadequate heat release (Nybo *et al.*, 2002), therefore this must be taken into account.

Heat induced fatalities (caused by high internal temperatures) are known to occur in athlete and military populations, whereby individuals push themselves to physical exertion during training and competitions in hot conditions (Gaoua *et al.*, 2011, Epstein *et al.*, 2012). Heat-stress exacerbates the development of fatigue (defined as the inability to maintain work at a given intensity) and thus performance in physical and cognitive tasks deteriorates at a faster rate than in temperate conditions (Nybo *et al.*, 2014). This early onset of fatigue is suggested to be a result of the complex interplay between various central and peripheral factors (Cheung and Sleivert, 2004, Nybo *et al.*, 2014). Understanding these factors and mechanisms and the way in which they are implicated in the fatigue process is crucial in the successful

development of interventions strategies to offset premature fatigue and alleviate heat-induced performance decrements. In a recent review, Nybo *et al.* (2014) present a detailed model illustrating how these factors integrate and collectively influence fatigue and ultimately performance (Figure 2.1). The present literature review will focus only on the relevant central, peripheral and psychological factors within this model.

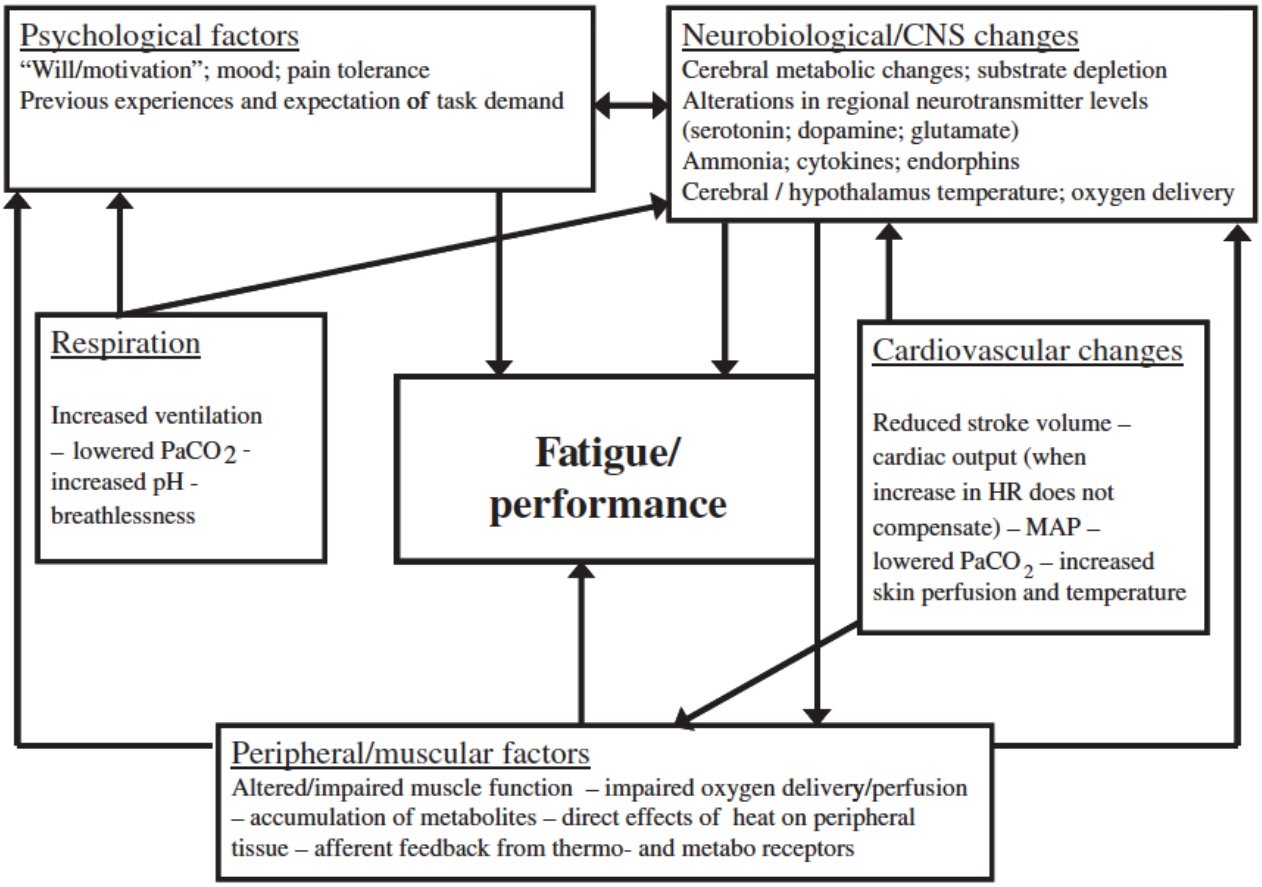

Figure 2.1. Integrative model incorporating cardiovascular, respiratory, CNS and peripheral factors that may contribute to exercise-induced fatigue in the heat (Nybo *et al.*, 2014).

2.3 Peripheral Aspects of Fatigue

Historically, the development of fatigue was primarily associated with peripheral factors, specifically the occurrence of a 'metabolic endpoint' (Meeusen *et al.*, 2006b).

These factors include, but are not limited to, substrate depletion, accumulation of metabolic by-products, adverse peripheral effects of hypohydration and an elevated core temperature (Roelands and Meeusen, 2010). These factors, individually and collectively contribute to the development of fatigue and thermal strain in both temperate and hot environments. However, as fatigue can arise without any signs of muscular dysfunction or inadequate availability of substrate, it is believed that mechanisms within the CNS are also implicated in the fatigue process (Foley and Fleshner, 2008). Therefore, fatigue should be acknowledged as 'complex phenomenon' occurring at all levels of the brain-muscle pathway (Roelands and Meeusen, 2010, Nybo *et al.*, 2014).

2.4 Central Fatigue

Centrally mediated fatigue has become a topic of interest over recent decades with several theories being suggested to elucidate the possible mechanisms involved (Nybo *et al.*, 2014). Alterations in cerebral temperature, perceived effort, motivation and monoamine neurotransmission are all suggested to be mechanistically associated with central fatigue in normal and hot environments (Foley and Fleshner, 2008). However, a specific definitive cause has not yet been discovered.

2.4.1 Critical Core Limit

It has long been recognised that a high core and brain temperature influence the ability and drive to continue exercising. Over recent years it has been demonstrated that voluntary exhaustion in the heat occurs at similar and consistent core body temperatures in humans (~39-40°C) (Gonzalez-Alonso *et al.*, 1999, Nybo and Nielsen, 2001a, Cheung and Sleivert, 2004). It is proposed that the notion of central fatigue is a protective mechanism against the attainment of a critically high core and brain temperature (~40°C), to prevent heat stroke and cell damage/death (Nielsen and Nybo, 2003, Nybo *et al.*, 2014). It has been suggested that above this 'critical core limit', humans are unable to continue exercising and thus a high internal temperature is considered the main limiting factor of performance in hot environments (Gonzalez-Alonso *et al.*, 1999). However, this theory should not be misconceived as if exhaustion/fatigue is dictated by a 'critical core temperate' threshold (Nybo *et al.*, 2014). Previous studies have demonstrated that pharmacological manipulation of DA

and administration of caffeine allows individuals to surpass this set 'limit' reaching higher core temperatures (~0.5°C) when exercising in the heat (Watson *et al.*, 2005a, Cheuvront *et al.*, 2009, Roelands and Meeusen, 2010, Nybo, 2012, Nybo *et al.*, 2014). Moreover, in competitions and training when athletes and military personnel are highly motivated and pushed to their limits, dangerously high core temperatures have been reached, exceeding those observed in laboratory conditions (Ely *et al.*, 2009, Epstein *et al.*, 2012). It is also evident that highly trained individuals are able to tolerate higher core temperatures (~39-40°C) before voluntary exhaustion, compared to their untrained counterparts (~38-39°C) (Cheung and Sleivert, 2004). Therefore, it is clear that core temperature is not solely responsible for hyperthermia-induced central fatigue and several other mechanisms within the CNS may also contribute to the fatigue response.

2.4.2 Neurotransmitter Systems

Several research groups have explored the theory that altered brain neurotransmitter concentrations may contribute to the fatigue process during exercise-heat-stress (Watson *et al.*, 2004, Hasegawa *et al.*, 2008, Roelands *et al.*, 2008, Roelands and Meeusen, 2010). It is recognised that specific monoamines can influence thermoregulation and that alterations in brain concentrations/ratios of 5-HT, DA and NA may play a key role in the premature onset of fatigue in hot environments (Roelands *et al.*, 2009, Meeusen and Roelands, 2010, Roelands and Meeusen, 2010).

2.4.2.1 The 'Central Fatigue Hypothesis'

Early links to the role of 5-HT and DA in centrally mediated fatigue initiated the development of the first neurotransmitter related theory, the 'central fatigue hypothesis' (Acworth *et al.*, 1986, Newsholme, 1987). This hypothesis is based on the concept that during prolonged exercise, the activity and synthesis of the central monoamines (5-HT, DA and NA) are altered (Meeusen *et al.*, 2006b). Specifically, it is suggested that increases in brain serotonergic activity during exercise may induce lethargy, losses in motivation and decreased mental alertness, consequently resulting in performance decline (Newsholme, 1987). An increase in brain 5-HT concentration is brought about by elevations in blood fatty acids during prolonged exercise as this promotes increases in free tryptophan, a precursor to 5-HT (Roelands and Meeusen,

2010). As tryptophan competes with branched-chain amino acids (BCAA) for transport through the BBB, an increased ratio favours the entry of tryptophan, which is then synthesised to 5-HT leading to higher brain concentrations (Meeusen *et al.*, 2006b). Despite the plausible theory behind the central fatigue hypothesis, evidence to support the role of 5-HT in centrally mediated fatigue in the heat is limited in humans (Roelands and Meeusen, 2010, Nybo *et al.*, 2014). Although, it is possible that dysregulation of 5-HT may act on other CNS systems (such as the dopaminergic system), indirectly influencing the development of fatigue during exercise (Meeusen *et al.*, 2006b, Nybo, 2010).

2.4.2.2 Dopamine and Central Fatigue

Several research groups have since challenged the original central fatigue hypothesis with the theory that dopaminergic activity and the interaction between brain 5-HT and DA also play an important role in CNS fatigue (Foley and Fleshner, 2008). As DA is associated with arousal, motivation, reward and attention, its involvement in central fatigue becomes obvious (Meeusen *et al.*, 2006b). The first links to DA in central fatigue came from early work on animals, demonstrating increases in dopaminergic activity during prolonged exercise (Bliss and Ailion, 1971), which may be relevant to physical and cognitive performance (Davis and Bailey, 1997). Furthermore, manipulation of brain DA concentrations with certain drugs (e.g. amphetamines) confirmed its involvement in the development of exercise-induced central fatigue (Gerald, 1978, Heyes *et al.*, 1985). Thus, on the basis of such observations, Newsholme's original 'central fatigue hypothesis' was adapted by Davis and Bailey (1997) to include the influence of DA. The revised hypothesis proposes that an increased brain ratio of 5-HT:DA is related to feelings of lethargy and tiredness, accelerating the fatigue process, while low ratios augment performance by preserving levels of motivation and arousal (Davis and Bailey, 1997).

Dopaminergic cells located in the mid-brain are the main source of DA within the CNS, found in the pars compacta region of the substantia nigra of the brain (Foley and Fleshner, 2008). The nigrostriatal pathway, involving cell bodies in the substantia nigra and striatum, is implicated in motor control and movement. Another important pathway is the mesocortical pathway, which connects the ventral tegmental area to the prefrontal cortex and is involved in normal cognitive control, motivation and

behavioural responses (Savitz *et al.*, 2006). Both the striatum and prefrontal cortex require dopaminergic projections to function and thus if DA synthesis is limited, declines in cognitive function will occur (Cools, 2008). The rate-limiting step in the biosynthesis of DA (and NA) is the hydroxylation of the amino acid TYR. As DA cannot readily cross the BBB, neurons must synthesis it in the brain themselves and the rate of synthesis depends upon the availability of TYR, a DA precursor (Foley and Fleshner, 2008). To enter the brain, TYR must compete with other LNAA, including tryptophan, to cross the BBB as they share the same transporter system (Cools, 2008). On reaching the presynaptic neuron, TYR is oxidised and converted to L-3-4-dihydroxyphenylalanine (L-Dopa) by the rate-limiting enzyme TYR hydroxylase. L-Dopa is subsequently decarboxylated via L-aromatic amino acid decarboxylase to form DA (Foley and Fleshner, 2008). In neurons that utilise DA as a transmitter, no further enzymatic pathways occur; however, neurons that use NA as a transmitter require an extra step to convert DA to NA via the enzyme DA-β-hydroxylase (Fernstrom and Fernstrom, 2007). Figure 2.2 provides a diagrammatic portrayal of the TYR to DA synthesis pathway.

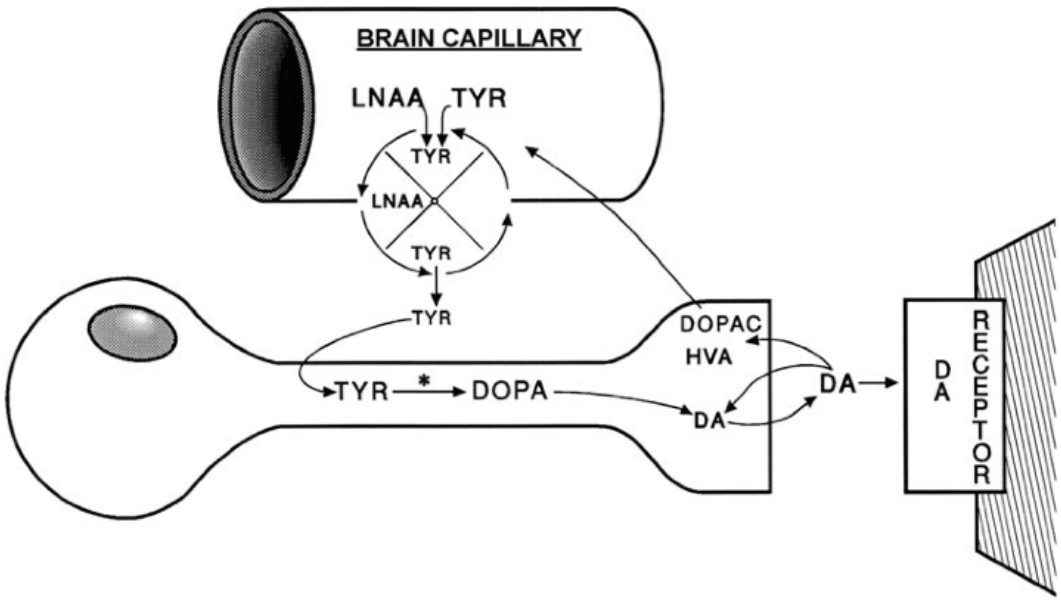

Figure 2.2. Simplified illustration of the competitive transport of TYR into the brain and the DA synthesis pathway in the presynaptic neuron. *Rate limiting enzyme tyrosine hydroxylase converts TYR to L-DOPA. DA is then metabolised to dihydroxyphenylacetic acid (DOPAC) and can be further catabolised to homovanillic acid (HVA) (Fernstrom, 2013).

After the conversion of TYR to DA through the above pathway, DA is released from the presynaptic terminal to activate G protein-coupled DA receptors (namely D_1 and D_2 class). Activation of these receptors occur from the binding of DA after it a crosses the inter-synaptic space (Cropley *et al.*, 2006). D_1 class receptors (D_1 and D_5) stimulate cyclic adenosine monophosphate (cAMP) production by adenylyl cyclase and are located postsynaptically on DA receptor cells. Whereas the D_2 class receptors (D_2, D_3 and D_4) induce inhibition of adenylyl cyclase and are expressed both postsynaptically (DA target cells) and presynaptically (DA neurons). In the brain, D_1 and D_2 receptors are expressed in both the striatum and frontal cortex (Beaulieu and Gainetdinov, 2011).

As previously discussed, during prolonged exercise, particularly in high ambient temperatures, there is a specific demand for DA to enable the maintenance of arousal, motivation (drive to continue exercise) and motor control. Initially, catecholamine synthesis and release is increased during stress (e.g. exercise and or/heat-stress) (Lieberman *et al.*, 2005). However, as exercise continues and exhaustion begins to occur, there is a marked decrease in central DA concentration (Wurtman *et al.*, 1980), therefore suggesting that depletion of central catecholamines may be associated with fatigue and performance in the heat (Bailey *et al.*, 1993, O'Brien *et al.*, 2007). This has been confirmed in animal models (rodents) as central DA depletion was shown to accelerate exhaustion, while an increase in dopaminergic activity prolonged time to exhaustion (Heyes *et al.*, 1985). Furthermore, a follow up study observed no influence of striatal DA depletion on exercise performance in the first 40% of an exhaustion run, however performance rapidly deteriorated during the latter 60% (Heyes *et al.*, 1988). These results indicate that during the latter stags of exercise, when exhaustion is imminent, there is a need to increase DA activity to improve exercise performance (Meeusen and Demeirleir, 1995).

2.4.2.3 Other Factors Influencing Central Fatigue

The notion of central fatigue is multifaceted and complex and there are various other factors that may influence the fatigue process. Not all of these factors have been explored in great detail and thus it is difficult to ascertain their exact contribution to central fatigue. Aside from the influence of the specific monoamines previously discussed, other neurotransmitters may also be involved in the fatigue process.

Gamma-aminobutyric acid (GABA) and glutamate have each been suggested to have a small involvement in the development of central fatigue (Meeusen *et al.*, 2006a). Glutamate is suggested to be the main excitatory neurotransmitter and GABA is the main inhibitory neurotransmitter in the mammalia cortex and thus have both been associated with exercise and central fatigue (Guezennec *et al.*, 1998). Another excitatory neurotransmitter that has been implicated in the fatigue process is acetylcholine. The synthesis, release and reuptake of acetylcholine is fundamental in the generation of muscular force and its association with memory, awareness and thermoregulation highlights its probable link with central fatigue within the CNS (Davis and Bailey, 1997). Furthermore, it is suggested that adenosine plays a role within the development of central fatigue as it inhibits the release of excitatory neurotransmitters such as DA and NA, consequently reducing arousal. The relationship between adenosine, caffeine and fatigue will be discussed in 2.6.2.

Another area of CNS fatigue that has received little attention is the BBB and how changes in its integrity may influence the fatigue process. The BBB is generally resistant to changes in permeability, however in some circumstances its function may be acutely or chronically compromised resulting in a disturbance of its mechanistic properties (Meeusen *et al.*, 2006a). To support this statement, there is evidence that prolonged exercise in a warm environment results in an increase in BBB permeability in humans (Watson *et al.*, 2005b). One factor that may contribute to increased BBB permeability is increased levels of ammonia that occur during exercise, which also influences cerebral levels of GABA and glutamate (Davis and Bailey, 1997). Furthermore, insulin plays a major regulatory role in the CNS and has several effects on barrier cell function (Banks *et al.*, 2012). Insulin enhances the transport of the amino acids TYR and tryptophan from the blood to the brain indirectly, by reducing the levels of the other BBB competing amino acids (Tagliamonte *et al.*, 1976). This results in an increased brain concentration of either TYR or tryptophan and thus may potentially influence central fatigue. Each of the factors outlined above may each influence the central fatigue process, however, the extent to which is currently unknown (Meeusen *et al.*, 2006a).

2.5 Heat-stress and Performance

As previously mentioned in section 2.2 of this review, prolonged exposure to hot environmental conditions impairs performance in both physical and cognitive tasks. Optimal performance (physical and cognitive) is of great importance to athletes and military personnel alike and even small decrements could significantly alter the outcome of competitions and military operations and in extreme circumstances, could be life threatening. A plethora of research has investigated the effects of heat-stress on exercise performance, whereas its effects on cognitive function have received significantly less attention (Hocking *et al.*, 2001).

2.5.1 Physical Performance

During physical activity in hot environments, the combination of endogenous heat production and exogenous heat-stress deteriorate performance. According to Nybo *et al.* (2014), this impairment is observed during both high-moderate intensity exercise and fixed and self-paced exercise and was present in 23 of the 24 studies examined within their review. Specific studies directly investigating the influence of heat-stress on exercise performance share comparable observations. For example, it has been demonstrated that high ambient temperatures (31°C) can reduce the capacity to perform prolonged physical exercise by ~50% when compared to a cooler environment (11°C) (Galloway and Maughan, 1997). Similarly, Tatterson *et al.* (2000) observed a 6.5% reduction in power output during a 30 min cycling time-trial in the heat (32°C), accompanied by a significant increase in skin temperature and sweat rate. Interestingly, core temperature was similar in both the hot and temperate condition in this investigation (23°C). More recently, performance in a pre-loaded time-trial (15 min) decreased as subjects covered less distance in the hot (30°C) compared to moderate condition (14°C) (Tyler and Sunderland, 2008). Furthermore, it has been shown that as environmental temperature increases there is usually a subsequent increase in rating of perceived exertion and thermal sensation for the exercise/task completed (Maw *et al.*, 1993, Nybo and Nielsen, 2001b). This supports the notion that internal temperature is not the only factor to influence fatigue in the heat as alterations in the subjective state of the individual may also play a part. It is suggested that the ‘drive’ to continue exercising is influenced by central mechanisms and is associated with exhaustion in the heat (Roelands and Meeusen, 2010).

In summary, it is clear that heat-stress (25-40°C) impairs exercise performance during a diverse range of exercise tasks (time-trial, exercise to exhaustion, pre-loaded exercise) and modes (cycling, treadmill running). Higher ambient temperatures and humidity have an exacerbated negative impact on performance as fatigue develops at an accelerated rate. Knowledge of the various mechanisms that influence fatigue has enabled the development of intervention strategies to alleviate heat-induced decrements in performance which are of great interest to athletes and individuals undertaking occupational tasks in extreme environments (Watson, 2008).

2.5.2 Cognitive Function

As previously discussed, exercise-heat-stress results in a number of physiological, thermal and perceptual changes, which may deter performance in cognitive tasks. However, compared to the physiological effects of heat-stress, its impact on cognitive function is not well understood (Hocking *et al.*, 2001, Gaoua, 2010). From the current literature, it is difficult to compare findings from previous studies assessing cognitive function due to the various different experimental designs employed (Gaoua, 2010). It is suggested that factors such as the type and complexity of the cognitive test, severity of heat exposure (temperature, duration), protocol design (passive or active heat-stress) and prior experience of the individual each influence the outcome measures and thus a complete systemic review of the literature is not possible (Hancock and Vasmatzidis, 2003, Gaoua *et al.*, 2011). This review will mainly focus on exercise-heat-stress (active heat exposure) based assessments of cognitive function.

Acute exercise (low-moderate intensity) has been shown to improved performance in both simple and complex cognitive tasks (Chmura *et al.*, 1998, Collardeau *et al.*, 2001), suggested to result from increases in arousal, cerebral blood flow and neurotransmitter release, occurring at the onset of exercise (Grego *et al.*, 2004). Therefore, it is possible that studies using exercise-heat-stress designs to induce hyperthermia-related fatigue may actually be blunting the negative effects of heat through the beneficial effects of exercise (Gaoua, 2010). However, this may not apply to prolonged exercise-heat-stress, which has been shown to impair cognitive function due to the fatiguing effects of both prolonged heat exposure (30-40°C) and long-duration exercise (Cian *et al.*, 2001, Hocking *et al.*, 2001, Bandelow *et al.*, 2010,

Watson *et al.*, 2012). Thus, the effect of exercise on cognitive function is dependent upon the magnitude of stress imposed on the individual.

Previous studies have utilised a wide variety of cognitive tasks with differing complexities, which adds to the difficulty in comparing and understanding findings in this area (Gaoua, 2010). It is generally accepted that heat-induced cognitive declines are task dependent, as complex tasks (i.e. working memory, vigilance, dual-task) are typically affected more by heat-stress than simpler tasks (i.e simple reaction time) requiring less attention (Hancock and Vasmatzidis, 2003, Gaoua, 2010, Gaoua *et al.*, 2011). This is supported by previous exercise-heat-stress studies in which decrements were observed in certain aspects of cognitive function but not others. For example, Watson *et al.* (2012) observed a significant increase in error rate during the complex component of the Stroop test after cycling to exhaustion in a hot environment (30°C; 50% RH); however reaction time and rapid visual information processing were not influenced. Furthermore, two studies demonstrated declines in other complex aspects of cognitive function (working and long term memory) after exercising in the heat (45-50°C and 35°C respectively) (Cian *et al.*, 2001, Hocking *et al.*, 2001). Similarly, a recent well-controlled, passive heat investigation found significant impairments in working memory without any alterations in simple attentional processes after exposure to extreme heat (50°C) and thus concluded that the effects of hyperthermia on cognitive function are task dependent (Gaoua *et al.*, 2011).

The mechanisms by which heat-stress impairs cognitive function are not completely clear, thus recent studies have attempted to elucidate this with the use of more direct measurements of brain function (Gaoua, 2010). Various brain imagining techniques have been utilised in relation to heat-stress, such as functional magnetic resonance imaging (fMRI) (Liu *et al.*, 2013), electroencephalography (EEG) (Hocking *et al.*, 2001, Sun *et al.*, 2011, Kishore *et al.*, 2013) to reveal functional areas of the brain responsible for specific cognitive processes. However, many of the studies utilising such advanced techniques were conducted during passive heat-stress due to the difficulty implementing these tools during exercise. One exercise-heat-stress study employed a specific EEG paradigm referred to as steady-state probe topography (SSPT), which can highlight the different relationships of the brains electrical activities during completion of cognitive tasks using steady-state visually evoked

potentials (Hocking *et al.*, 2001). Under thermally stressful conditions they observed increased amplitude and decreased latency in the frontal and occipitoparietal regions of the brain, indicating greater use of the neural resources to sustain performance in the heat. Additionally, Nielsen *et al.* (2001) investigated the EEG activity of the frontal cortex during exercise in the heat and observed an increase in activity at the onset of exercise, followed by supressed arousal in the hot conditions compared to control. This suggests that electrical activity alterations in the brain's frontal cortex occurred as a result of hyperthermia-induced fatigue (Nielsen *et al.*, 2001, Gaoua, 2010).

Although the evidence is not entirely clear due to methodological variances, it appears that exercise-heat-stress negatively affects cognitive function, especially performance in complex tasks (Hocking *et al.*, 2001, Hancock and Vasmatzidis, 2003, Gaoua, 2010). A variety of factors influence cognitive processes during exercise-heat-stress, hence there is no stand-alone mechanism reported in the literature. An increase in internal temperature is suggested to alter electrical activity within certain brain regions, suppressing arousal levels (Nielsen *et al.*, 2001). Elevations in skin temperature (~3°C) have also been shown to influence cognitive function by altering the subjective state of individuals and increasing sensory displeasure (Gaoua *et al.*, 2012). Furthermore, as previously mentioned, alterations in the activity and synthesis of central monoamines, namely DA, NA and 5-HT, may induce declines in cognitive function due to their association with motor control and alertness. Specifically, as previously mentioned, exercise in the heat demands a constant supply of DA to maintain levels of motivation, arousal and motor control, compared to temperate conditions (Meeusen, 2014). Each of these factors play a role in hyperthermia-induced fatigue and contribute to cognitive decline during exercise-heat-stress (Nybo *et al.*, 2014), therefore interventions strategies that can alleviate such declines may be beneficial to performance.

2.6 Nutritional Manipulations

Events arising entirely within the CNS can influence fatigue (Watson, 2008) as described in sections 2.4.2 and 2.5.2. Alterations in the ratio of 5-HT:DA is implicated in the fatigue process, with high ratios inducing lethargy and motivation

losses and low ratios favouring performance improvements (Meeusen *et al.*, 2006b). This knowledge proffers the opportunity to manipulate the CNS in favour of enhanced performance with the use of nutritional interventions strategies (Roelands and Meeusen, 2010). Dietary mediated improvements may be beneficial to individuals working and exercising in hot environments for performance and safety purposes, hence the efficacy of numerous nutrients and supplements have been assessed, namely BCAA, carbohydrates, caffeine and TYR (Watson, 2008). Table 2.1 provides an overview of the neurotransmitters involved in the development of fatigue and the proposed effect of specific nutritional supplements used to manipulate them, which will be discussed in detail in further sections.

Table 2.1. Overview of the main neurotransmitters involved in the fatigue process and the nutritional supplements used to manipulate them. Adapted from Watson (2008).

Neurotransmitter	**Role(s)**	**Manipulation**
Serotonin (5-HT)	Involved in feelings of tiredness, lethargy and sleep. Also influences pain, appetite regulation and thermoregulation	Tryptophan: ↑ 5-HT BCAA: ↓ 5-HT Carbohydrate: ↓ 5-HT
Dopamine (DA)	Plays an important role in motivation, memory, reward, attention and motor control and thermoregulation	TYR: ↑ DA BCAA: ↓ DA (?)
Noradrenaline (NA)	Involved in the regulation of attention, arousal and also learning and memory, anxiety, pain and mood	TYR: ↑ NA BCAA: ↓ NA (?)
Adenosine	Acts as both a neurotransmitter and a neuromodulator and is able to inhibit excitatory neurotransmitters, namely DA and NA	Caffeine: ↓ adenosine

2.6.1 Manipulation of 5-HT

Many previous studies have attempted to manipulate central levels of 5-HT with supplementation of specific nutrients, such as amino acids (BCAA) and carbohydrates (Meeusen *et al.*, 2006b, Fernstrom, 2013). Ingestion of both BCAA and carbohydrates are suggested to lower the ratio of free tryptophan:BCAA (via an increase in plasma BCAA availability and suppression of lipolysis respectively), which reduces its

transport across the BBB, thus limiting 5-HT synthesis in the brain (Davis *et al.*, 1992, Meeusen *et al.*, 2006b). It is hypothesised that reducing cerebral 5-HT concentrations will attenuate feelings of fatigue and enhance performance (Table 2.1). However, despite the plausible mechanism for the use of such nutrients, there is limited evidence to support this, especially in hot environments (Meeusen, 2014). Specifically, BCAA ingestion did not improve time to exhaustion (Watson *et al.*, 2004), time-trial performance (Cheuvront *et al.*, 2004) or cognitive function (Mittleman *et al.*, 1998, Cheuvront *et al.*, 2004) after moderate intensity exercise in the heat (30-40°C). A possible explanation for the failure to observe a benefit from BCAA ingestion may be related to its influence on DA synthesis. Administration of exogenous BCAA not only attenuates the rise in brain 5-HT but also reduces the uptake of TYR, which limits the synthesis and release of DA (Watson, 2008). Therefore, the negative effects associated with a decline in DA (Table 2.1) may counteract the positive effects of reducing 5-HT, consequently having no influence on physical or cognitive performance (Fernstrom, 2013). It is likely that this effect could be eradicated if the BCAA mixture also contained appropriate amounts of TYR, however this is yet to be investigated.

2.6.2 Manipulation of DA

Other research has focused on the nutritional manipulation of DA in an attempt to attenuate central fatigue and improve performance in physical and cognitive tasks (Table 2.1). Caffeine has been investigated due to its central effects as an adenosine antagonist whereby caffeine binds to adenosine receptors, blocking its activity and sedative-like action (Watson, 2008). Adenosine is capable of inhibiting the release of the excitatory neurotransmitters (DA and NA), thus ingestion of caffeine results in elevations in central DA and NA (Meeusen, 2014). However, evidence for a beneficial effect of caffeine during exercise in the heat is currently limited. A recent study demonstrated a ‘worthwhile’ improvement in cycling time-trial performance in a hot environment after administration of caffeine (35°C; 25% RH) (Pitchford *et al.*, 2014), whereas previous studies have reported no effect in similar environments on exercise performance (Cheuvront *et al.*, 2009, Roelands *et al.*, 2011) and cognitive function (Zhang *et al.*, 2014).

2.6.2.1 Tyrosine

The dietary precursor for catecholamine synthesis (DA and NA) is TYR, a non-essential amino acid contained within protein rich dietary sources and synthesised in the liver from phenylalanine (Wurtman *et al.*, 1980). Ingestion of exogenous TYR increases its ratio to other LNAA (including tryptophan) for competitive transport across the BBB (Figure 2.2), thus resulting in a greater cerebral uptake and an increase in DA synthesis in the brain (Fernstrom and Faller, 1978, Gibson and Wurtman, 1978). The synthesis of TYR to DA is controlled by a rate-limiting step, the conversion of TYR to L-DOPA via the enzyme TYR hydroxylase, as explained previously in section 2.4.2.2.

Under normal conditions in the absence of stress, levels of TYR hydroxylase are apparently saturated with substrate, thus exogenous supply of TYR should not in theory influence catecholamine synthesis in the brain (Foley and Fleshner, 2008). However, during periods of acute or chronic stress, catecholamine turnover is up-regulated with observed elevations in concentrations of catecholamine neurotransmitters in several brain regions including the striatum and hypothalamus of rodents (Lehnert *et al.*, 1984, Meeusen *et al.*, 1997, Foley and Fleshner, 2008). As more neurotransmitters are synthesised to meet the demands of the specific stressor, the precursor TYR is expended and thus availability becomes low (Jongkees *et al.*, 2015). At the point of exhaustion, brain tissue DA content is markedly decreased and thus it is acknowledged that depletion of brain catecholamines and TYR availability influences stress-induced fatigue (Bailey *et al.*, 1993, O'Brien *et al.*, 2007).

As the synthesis of DA and NA in the brain relies on the availability of their amino acid precursor TYR, some studies have investigated the effects of an amino acid mixture lacking both TYR and it's precursor phenylalanine (Harmer *et al.*, 2001). Such a mixture decreases the availability of TYR to the brain and increases the competition for its transport across the BBB. Animal studies have shown that administration of a TYR-free mixture decreases brain TYR and catecholamine synthesis (McTavish *et al.*, 1999), resulting in reduced locomotor responses (McTavish et al. unpublished data cited in Harmer *et al.* (2001)). Consistent with this, in humans TYR depletion was shown to affect DA function from a variety of different

measures (Harmer *et al.*, 2001). The TYR-free mixture resulted in higher prolactin levels, which indicates reduced dopaminergic function within the brain. Moreover, TYR depletion impaired aspects of cognitive function and it was also reported that individuals felt 'less good' after the TYR-free compared to balanced mixture. Together, these findings suggest that TYR availability may negatively influence DA function in humans and thus supports the rationale for supplementing TYR to increase catecholamine synthesis and release.

Since DA and NA have been associated with arousal, motivation, attention and motor control, several studies have utilised TYR as a nutritional supplement prior to extreme stress in an attempt to alleviate stress-related decrements in performance (physical and cognitive). It is suggested that similar to the effects of physical stress and heat-stress, catecholamine concentrations also become depleted during exposure to other environmental stressors (cold and hypoxia) (O'Brien *et al.*, 2007). The majority of literature assessing the effects of TYR is military based, with several investigations conducted by the US Army Research Institute (Banderet and Lieberman, 1989, Lieberman, 2003, Lieberman *et al.*, 2005, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007) and other army institutes (Deijen and Orlebeke, 1994, Deijen *et al.*, 1999). The highlighted military investigations have primarily focused on aspects of cognitive function (complex; working memory, vigilance, tracking and simple; reaction time) and mood during exposure to acute stress, such as such as cold (Banderet and Lieberman, 1989, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007) and hypoxia (Banderet and Lieberman, 1989), and paradigms involving both extended wakefulness (Neri *et al.*, 1995) and the physical/emotional stress nexus (Deijen *et al.*, 1999). Each of these aforementioned studies has demonstrated improvements in specific aspects of cognitive function after ingestion of TYR (100-300 $mg \cdot kg$ body $mass^{-1}$); refer to Table 2.2 for an overview of these military based findings. Moreover, specific to hot environments, it has been shown that TYR improves the behavioural response (ability to cope) to exercise-heat-stress (41°C) and increases NA release, albeit in rodents and not humans (Lieberman *et al.*, 2005).

On the basis of these positive findings, several further studies have investigated the effects of TYR in relation to exercise performance in normal (Strüder *et al.*, 1998, Chinevere *et al.*, 2002, Sutton *et al.*, 2005) and elevated/high ambient temperatures

(Tumilty *et al.*, 2011, Watson *et al.*, 2012, Tumilty *et al.*, 2014, Coull *et al.*, 2015) (see Table 2.2 for overview of these studies). All three of the studies conducted under temperate conditions failed to observe any beneficial effect of acute TYR ingestion on endurance performance (Strüder *et al.*, 1998, Chinevere *et al.*, 2002) or strength and power performance (Sutton *et al.*, 2005). These findings are not surprising due to the questionable amount of stress experienced during exercise in normal ambient temperatures and the relationship between stress and catecholamine turnover as previously mentioned.

Hence, all recent studies in this area have employed passive and active heat-stress based designs to examine the influence of TYR under extreme stress and attempt to elucidate its mechanistic properties. The first of these investigations reported a significant improvement in exercise capacity (15 ± 11%) after ingestion of 150 mg·kg body mass^{-1}, compared to placebo when cycling to exhaustion in a hot environment (30°C; 50% RH) (Tumilty *et al.*, 2011). To date, this is the first and only study to observe a positive effect of TYR on physical performance, despite the efforts of Watson *et al.* (2012) who attempted to replicate this study. Watson *et al.* (2012) reported that ingestion of TYR did not influence exercise capacity or any aspects of cognitive function (reaction time, information processing or memory) in the heat, despite a significant increase in plasma TYR concentration. Moreover, a follow up study by Tumilty *et al.* (2014) employed a pre-loaded time-trial design based on the theory that a benefit of TYR would be more apparent during self-paced exercise due to the greater influence of behavioural thermoregulation, motivation and arousal compared to constant load exercise (Nybo *et al.*, 2014, Tumilty *et al.*, 2014). However, this was not the case, as TYR ingestion (150 mg·kg body mass^{-1}) did not influence time-trial performance when performed in a hot environment (30°C; 50% RH). Very recently, Coull *et al.* (2015) examined the effect of TYR ingestion (300 mg·kg body mass^{-1}) during exposure to a 90 min soccer-simulation protocol (iSPT; (Aldous *et al.*, 2014)) in a warm environment (25°C; 40% RH). Interestingly, TYR had a positive effect on cognitive function (vigilance) and readiness to invest mental effort, but did not influence physical performance (distance covered during the soccer simulation).

Table 2.2. Overview of previous studies assessing the effects of TYR on cognitive function, exercise and a combination of both.

Study	TYR dose	Exposure/exercise	Blood samples	Cognitive function	Findings
Cognitive function only					
Banderet and Lieberman (1989)	2 x 50 mg/kg separated by 40 min (capsule)	Cold and hypoxic (15 °C/ 4700 m)	No	Reaction time & vigilance performance + mood states	↓ symptoms, adverse moods & performance impairments
Shurtleff *et al.* (1994)	150 mg/kg 2 h pre-exposure	Cold (4°C)	Yes	Working memory computer task	↑ working memory ↑ TYR, ↑ NE in cold
Neri *et al.* (1995)	150 mg/kg split dose 6 h into testing	24 h sleep deprivation	No	Vigilance, psychomotor tasks and mood states	↑ vigilance & psychomotor performance for 3 h
Deijen *et al.* (1999)	2 g/d over 5 days (protein rich drink)	1 week physical combat course	No	Memory, tracking, dual-task and mood states	↑ memory and tracking
O'Brien *et al.* (2007)	2 x 150 mg/kg separated by 4 h (energy bar)	Cold air + water immersion (10°C)	No	Memory, pattern recognition, reaction time and vigilance	↑ working memory & psychomotor tasks
Mahoney *et al.* (2007)	2 x 150 mg/kg separated by 4 h (energy bar)	Cold water immersion (10°C)	No	Working memory, reaction time, vigilance and mood state	↑ working memory & psychomotor tasks
Kishore *et al.* (2013)	6.5 g 90 min pre-exposure (energy bar)	90 min passive heat (45°C; 30% RH)	Yes	Reaction time, ERP and CNV	↑ plasma DA, NA, A ↑ cognitive function

Study	TYR dose	Protocol/exposure	Blood samples	Cognitive function	Findings
Exercise only					
Strüder *et al.* (1998)	2 x 10 g (drink)	Cycling to exhaustion	Yes	N/A	↑ plasma TYR ↔ perf
Chinevere *et al.* (2002)	6 x 25 mg/kg (drink)	90 min cycling bout + time-trial	Yes	N/A	↑ plasma TYR ↔ TT perf
Sutton *et al.* (2005)	150 mg/kg 30 min pre-exercise (apple sauce)	Load-carriage & phys perf battery	Yes	N/A	↑ plasma TYR and NA ↔ endurance/strength/power
Tumilty *et al.* (2011)	150 mg/kg 1 h pre-exercise (drink)	Cycling to exhaustion (30°C; 60% RH)	Yes	N/A	↑ plasma TYR ↑ of 15 ± 11% T_{ex}
Tumilty *et al.* (2014)	150 mg/kg 1 h pre-exercise (drink)	60 min cycling + time-trial (30°C; 60% RH)	Yes	N/A	↑ plasma TYR ↔ TT perf
Cognitive function & Exercise					
Watson *et al.* (2012)	150 mg/kg total in 30 min intervals pre-exercise (drink)	Cycling to exhaustion (30°C; 50% RH)	Yes	Stroop test, Sternberg memory & RVIP	↑ serum TYR ↔ T_{ex} or cognitive function
Coull *et al.* (2015)	2 x 150 mg/kg separated by 4 h (drink)	90 min soccer simulation (25°C; 40% RH)	No	Vigilance and dual-task (PsychE)	↔ physical perf ↑ vigilance and RTIME

↑ = increase; ↓ = decrease; ↔ = no change; perf = performance; TT = time-trial; T_{ex} = time to exhaustion.

From the available literature, it appears that TYR may not be useful in improving exercise performance in normal and hot conditions, however there is a large body of literature that support its use to improve aspects of cognitive function. A recent passive heat-stress investigation supports this statement after demonstrating that TYR alleviates heat-induced delays in reaction time during 90 min passive exposure to 45°C; 30% RH (Kishore *et al.*, 2013). This study also assessed higher levels of cognitive function using advanced brain imaging techniques (event related potentials; ERP), providing evidence that heat exposure causes an increase in P300 (reduced concentration) and M100 latency (reduced ability to react to a warning) and a decrease in M100 amplitude (linked with attention) which returned to near normal levels after ingestion of TYR. It was concluded that the higher DA and NA concentrations detected in the TYR trial might have maintained cognitive function by alleviating the decrements associated with heat-stress (Kishore *et al.*, 2013). To date, this is the only TYR-heat-stress based study to assess DA and NA concentrations in combination with cognitive testing and brain imaging and thus provides valuable insight into the understanding of the mechanistic action of TYR in response to heat-stress, which is an important advancement in this area.

The contrasting findings in the literature in relation to TYR ingestion may be a result of the various methodological differences among previous studies. It has been suggested that the magnitude of activation of the catecholamine system, subsequent to the stress induced by the different exposures and exercise protocols, may be a possible explanation (Tumilty *et al.*, 2014). As previously mentioned, highly stressful experiences increase catecholamine activity whereby there is a need for extra TYR supply, thus situations inducing more stress should hypothetically benefit from TYR ingestion (Lehnert *et al.*, 1984, Foley and Fleshner, 2008). Furthermore, it has been highlighted that the diverse range of administration strategies (dose, form and source of the supplement) utilised by previous studies (see Table 2.2 for specifics) may also contribute to the differing findings (Tumilty *et al.*, 2014, Coull *et al.*, 2015). To assess the efficacy of TYR as an ergogenic aid, future studies must standardise these factors to enable appropriate conclusions to be made with minimal confounding variables. Investigation into the pharmacokinetics of TYR ingestion is also recommended before further experimental studies are conducted to identify an ‘optimal’ dose (Coull *et al.*, 2015).

Data regarding the pharmacokinetics, or dose response relationships of TYR ingestion are currently limited. Lack of information in this area makes it difficult to identify the optimal dose and time to ingest TYR prior to exercise or stressful exposure. Not all studies assessing the effects of TYR on performance aspects have reported plasma/serum measures of TYR and amino acid concentrations (see Table 2.2). To date, only one previous study has directly investigated the pharmacokinetics of two separate doses of TYR (100 and 150 mg·kg body mass^{-1}) and reported dose related increases in plasma TYR whereby the 150 mg·kg body mass^{-1} dose provided the highest elevation (Glaeser *et al.*, 1979). Furthermore, it was observed that TYR concentration in the blood peaked 2 h post ingestion of the supplement. However, this study provides very basic details and only assessed small doses of TYR, thus further, updated research is required due to the popularity of TYR in research and in the field. The most commonly administered acute doses within the literature are 150 (Neri *et al.*, 1995, Sutton *et al.*, 2005, Tumilty *et al.*, 2011, Watson *et al.*, 2012, Tumilty *et al.*, 2014) and 300 mg·kg body mass^{-1} TYR (ingested in a double dose of 150 mg·kg body mass^{-1} TYR 4 h apart) (Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Coull *et al.*, 2015), hence it would be appropriate to investigate the direct effects of these doses on TYR concentrations in the blood.

2.7 Overall Summary

It is evident that exercise in hot environments accelerates the development of fatigue, due to a number of contributing factors, consequently deterring performance in physical and cognitive tasks (Nybo *et al.*, 2014). Specifically, one contributing factor is the influence of neurotransmitters on the fatigue process. It has been highlighted in animal models that exposure to stress leads to an increase (Meeusen *et al.*, 1997) and subsequent depletion in NA and DA levels with performance decreasing accordingly (Lehnert *et al.*, 1984). Ingestion of TYR, a catecholamine precursor, has shown some evidence of beneficial effects on cognitive function during stress (Banderet and Lieberman, 1989, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Kishore *et al.*, 2013, Coull *et al.*, 2015) but evidence of a positive influence on physical performance in limited to one study (Tumilty *et al.*, 2011). Methodological inconsistencies in the literature may be a possible explanation for some of the opposing findings due to a diverse range of protocols, stressful exposures, and TYR administration strategies.

Before any further experimental studies are conducted, investigation into the required dose of TYR to elicit significant elevations in blood TYR concentrations is necessary (Coull *et al.*, 2015). Furthermore, as numerous military operations are conducted overseas in hot environmental conditions, investigation into the effect of TYR under similar conditions may be useful due to the popularity and success of TYR in previous military based studies. Optimal performance in such conditions is paramount as heat-induced errors in judgement and decision making may have severe consequences on the health and safety of individuals, particular military personnel (Gaoua *et al.*, 2011). Thus any small dietary mediated improvements in performance may be of great benefit to military personnel.

2.8 Aims and Hypotheses

Experimental Chapter 1

To investigate the effects of acute TYR ingestion strategies (0, 150 and 300 mg·kg body mass^{-1} TYR administered in 2 equal doses, 4 h apart) on serum TYR concentrations at rest.

- Ingestion of 150 and 300 mg·kg body mass^{-1} TYR will significantly increase serum concentrations of TYR compared to control.

- Ingestion of 300 mg·kg body mass^{-1} TYR will induce significantly higher peak concentrations of TYR compared to 150 mg·kg body mass^{-1}.

Experimental Chapter 2

To investigate the effects of the 'optimal' TYR dose (ascertained from experimental chapter 1) on steady state exercise, cognitive function and time-trial performance during a military based exercise protocol in the heat (40°C; 30% RH).

- Ingestion of 150 mg·kg body mass^{-1} TYR will significantly improve aspects of cognitive function.
- Ingestion of 150 mg·kg body mass^{-1} TYR will not influence time-trial performance.

CHAPTER 3: General Methodology

This chapter outlines the general procedures employed within the studies described in experimental chapters 1 and 2. Schematics are provided within each experimental chapter for an overview of each study design and experimental protocol.

3.1 Participants

Upon ethical approval from the Institute for Sport and Physical Activity Research (ISPAR) Ethics Committee (approval no. 2013SPA006) healthy, recreationally active (5-10 $h \cdot wk^{-1}$ physical activity) males were recruited via convenience sampling. Prior to participation, a detailed information sheet (Appendix A) outlining the purpose, procedures and potential risks of the study was provided to each participant and also verbally explained by the researcher. Participants were able to ask any questions regarding their involvement in the study and were fully aware that they could withdraw at any time, without disadvantage or explanation. Subsequently, written informed consent was obtained in line with the principles stated in the Declaration of Helsinki (Appendix B). To minimise potential risks to themselves and the researcher, participants were also medically screened via a blood analysis (Appendix C), PAR-Q, and risk assessment questionnaire (Appendix D). All participants were non-smokers, free from any known medical condition, and musculoskeletal injury. Participants were excluded from the study if it was deemed unsafe for them to participate (Appendix A and C for risks associated with participating).

Written pre-test instructions (Appendix E) were provided, and participants were verbally briefed on these prior to testing. Participants were required to refrain from alcohol, caffeine, strenuous exercise and exposure to extreme environments (i.e. heat or hypoxic) for 24 h prior to each laboratory visit (Tumilty *et al.*, 2011, Watson *et al.*, 2012, Coull *et al.*, 2015). Additionally participants abstained from ingestion of any supplements (including protein, BCAA and caffeine) and did not take part in any other research projects involving supplementation of any kind for at least four weeks prior to testing. Participants were not heat acclimated or acclimatised prior to taking part in the experiments and study 2 (heat-based) took place in UK autumn and winter months. Each participant completed 24 h food diaries throughout the study to enable them to replicate their diet on subsequent visits (Tumilty *et al.*, 2011, Watson *et al.*,

2012, Coull *et al.*, 2015). Furthermore, participants were instructed to drink 500 mL of water prior to arriving at the laboratory in line with the American College of Sports Medicine Position Stand (Sawka *et al.*, 2007). Upon arrival, participants ingested a further 250 mL of water, before providing a urine sample for assessment of hydration status via a urine refractometer (Pocket Pal-Osmo, Atago Vitech Scientific, HAB Direct, UK). Samples were assessed in duplicate and participants were deemed euhydrated if urine osmolality was <600 $mOsm \cdot Kg^{-1}$ H_2O (Hillman *et al.*, 2011, Hillman *et al.*, 2013). All pre-experimental controls were monitored via a pre-test questionnaire (Appendix F), with apparent adherence confirmed at 100% in all instances. Data and information collected about the participants was stored on a password-protected laptop, which only the primary researcher had access to. No reference was made to individual participants to ensure anonymity and confidentiality was preserved.

3.2 Anthropometric Data

Anthropometric data was collected during the initial visit upon arrival at the laboratory. Body mass (kg) and height (cm) were recorded using Digital Tanita weighing scales, accurate to the nearest g (BWB0800, Allied Weighing, UK) and a Stadiometer, accurate to the nearest mm (Holtain Ltd, UK), respectively. Body fat percentage (%) was also assessed via Air Displacement Plethysmography (BodPod 2000A, Cranlea, UK) on a subsequent day in line with manufactures instructions, in a fasted state (no food/fluid for 4 h) with an evacuated bladder.

3.3 Tyrosine supplementation

The TYR supplement (L-Tyrosine) was sourced from an Advanced Medical Nutrition company (Nutricia Ltd, UK) in line with Watson *et al.* (2012). The purity of this supplement was assessed via high-performance liquid chromatography (HPLC) and confirmed to contain a satisfactorily high concentration of TYR (>98%). For the purpose of this research the supplement was added to a drink (a liquid suspension was formed) [250 mL sugar free lemon squash (Tesco, UK) and water] and orally ingested by participants in a double-blind manner, in line with previous research (Tumilty *et al.*, 2011, Watson *et al.*, 2012, Tumilty *et al.*, 2014, Coull *et al.*, 2015). To ensure the TYR ingestion was double-blinded, all drinks were provided in opaque sports bottles

and shaken vigorously immediately before ingestion to disguise the substance and were indistinguishable in taste and texture to the participants. Each drink was measured using the same weighing scales, accurate to the nearest 0.01 mg (Mettler, AT250). The specific dose of TYR administered is stated in each respective experimental chapter.

3.4 Statistical Analyses

Power calculations (G*Power 3) were conducted for both studies to identify the number of participants required to obtain a statistical power of 99%, at an alpha level of 0.05. All data was analysed using the statistical software package IBM SPSS statistics for Macintosh, Version 20 (IBM Corp, Armonk, NY). Statistical assumptions were assessed using conventional graphical methods (Grafen *et al.*, 2002) and deemed plausible for each variable. Quantile-quantile (Q-Q) plots were used to assess the normality of distribution of all data collected and was deemed plausible for each variable. Linear mixed models were used to identify differences in the dependant variables between groups (group x time) and conditions (condition x time) in experimental chapter 1 and 2, respectively (see section 4.3.7 and 5.3.6 for details). Where significance was obtained, Sidak post-hoc tests were used to locate significant pairs. Linear mixed models where chosen as this type of analysis permits missing data and can identify the most appropriate covariate structure for repeated measures data. Although there were very few missing data (only in study 1 with 3 missing blood samples), this analysis was preferred. The most suitable covariate model was decided using the likelihood variable test, which uses the $\chi 2$ critical test statistic to select the best fitting model, based on the change in the -2 restricted log likelihood of the two models. Furthermore, normality and homogeneity of variance of the residuals were assessed using Q-Q plots and scatter plots respectively, and were deemed plausible in each instance. Two-tailed statistical significance was accepted at the $p < 0.05$ level.

CHAPTER 4: Experimental Chapter 1 – Serum Response of Acute Tyrosine Ingestion at Rest

4.1 Introduction

Prolonged exposure to hot environments can accelerate the onset of fatigue, compromising performance in both physical (González-Alonso *et al.*, 1999, Nybo *et al.*, 2014) and cognitive tasks (Maughan *et al.*, 2007, Simmons *et al.*, 2008, Gaoua *et al.*, 2011). Many interventions to alleviate fatigue and performance decline in stressful environments have been investigated, including various nutritional manipulation strategies (Roelands and Meeusen, 2010, Meeusen, 2014). The amino-acid TYR, first investigated in a performance setting by the US Army Research Institute (Banderet and Lieberman, 1989, Lieberman *et al.*, 2005, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007), has seen several studies examining its effects on exercise performance (Tumilty *et al.*, 2011, Watson *et al.*, 2012, Tumilty *et al.*, 2014, Coull *et al.*, 2015) and cognitive function (Watson *et al.*, 2012, Coull *et al.*, 2015, Tumilty *et al.*, 2014).

Despite the previous positive military based findings (Banderet and Lieberman, 1989, Deijen *et al.*, 1999, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007), there are currently mixed opinions on the efficacy of TYR as an ergogenic aid, due to recent opposing findings (Watson *et al.*, 2012, Tumilty *et al.*, 2014). This inconsistency may be a result of the varying administration strategies and the TYR supplement itself (composition, purity, etc.). The most commonly administered dose within the literature is between 150 and 300 mg·kg body mass^{-1} TYR, typically ingested 1 h pre-exercise (Tumilty *et al.*, 2011, Tumilty *et al.*, 2014) or in two separate doses with a 4 h loading phase (Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Coull *et al.*, 2015). Lack of data on the blood response to TYR ingestion restricts its use as a nutritional aid due to limited knowledge on dose response relationships (Marriott and Thomas, 1994). Thus, data on dosing strategies (relative to peak plasma/serum TYR) would provide a standardised prescription for future research to utilise when investigating the effects of TYR (Tumilty *et al.*, 2014, Coull *et al.*, 2015). To the authors knowledge only one study has directly investigated this using 100 and 150 mg·kg body mass^{-1} at rest, providing very basic information on small doses of TYR (Glaeser *et al.*, 1979).

4.2 Aim and Hypotheses

The purpose of this study was to investigate the effects of acute TYR ingestion strategies (0, 150 and 300 mg·kg body mass^{-1} TYR administered in 2 equally split doses, 4 h apart) on serum TYR concentrations at rest. It was hypothesised that the 150 and 300 mg·kg body mass^{-1} TYR doses would significantly increase serum concentrations of TYR compared to control. Secondly, it was hypothesised that ingestion of 300 mg·kg body mass^{-1} TYR would induce significantly higher peak concentrations of TYR compared to 150 mg·kg body mass^{-1}.

4.3 Methodology

4.3.1 Participants

Twenty-one healthy, healthy males volunteered to participate in this study (median: age 21 y, range: 19-26 y, mean ± SD: height 178.4 ± 7 cm, body mass 76.3 ± 10.3 kg, body fat 14.3 ± 3.6%, activity level 6.7 ± 2 $h \cdot wk^{-1}$) (see table 4.1 for group descriptives). Inclusion and exclusion criteria is detailed in section 3.1.

Table 4.1. Participant anthropometric data in each group. Data is mean ± SD.

Measure	**CON (n = 7)**	**LOW (n = 7)**	**HIGH (n = 7)**
Age (y)	21 ± 1	22 ± 2	21 ± 2
Height (cm)	181.4 ± 7.5	176.2 ± 7.7	177.7 ± 5.8
Mass (kg)	79.9 ± 10.8	73.2 ± 11.9	75.7 ± 8.2
Body fat (%)	15.1 ± 3.2	13.9 ± 4	13.9 ± 4

4.3.2 Experimental Design

This study employed a double-blind, randomised, independent group design (see Figure 4.1 for study schematic) whereby each participant was randomly assigned (by a separate laboratory technician whom was not involved in the study) to one of three groups (n = 7 per group); control (CON; placebo - no TYR), low dose (LOW; 150 $mg \cdot kg$ body $mass^{-1}$ TYR) or high dose (HIGH; 300 $mg \cdot kg$ body $mass^{-1}$ TYR). Participants remaining within the laboratory for a period of 9 h (0800 until 1700). During this time, blood was drawn every hour, as described in further detail below in section 4.3.4. TYR was ingested in two separate doses, separated by a 4 h loading phase, at both 0900 and 1300 (see section 4.3.6 for further details). Standardised breakfast and lunch were provided at 0800 and 1200 respectively.

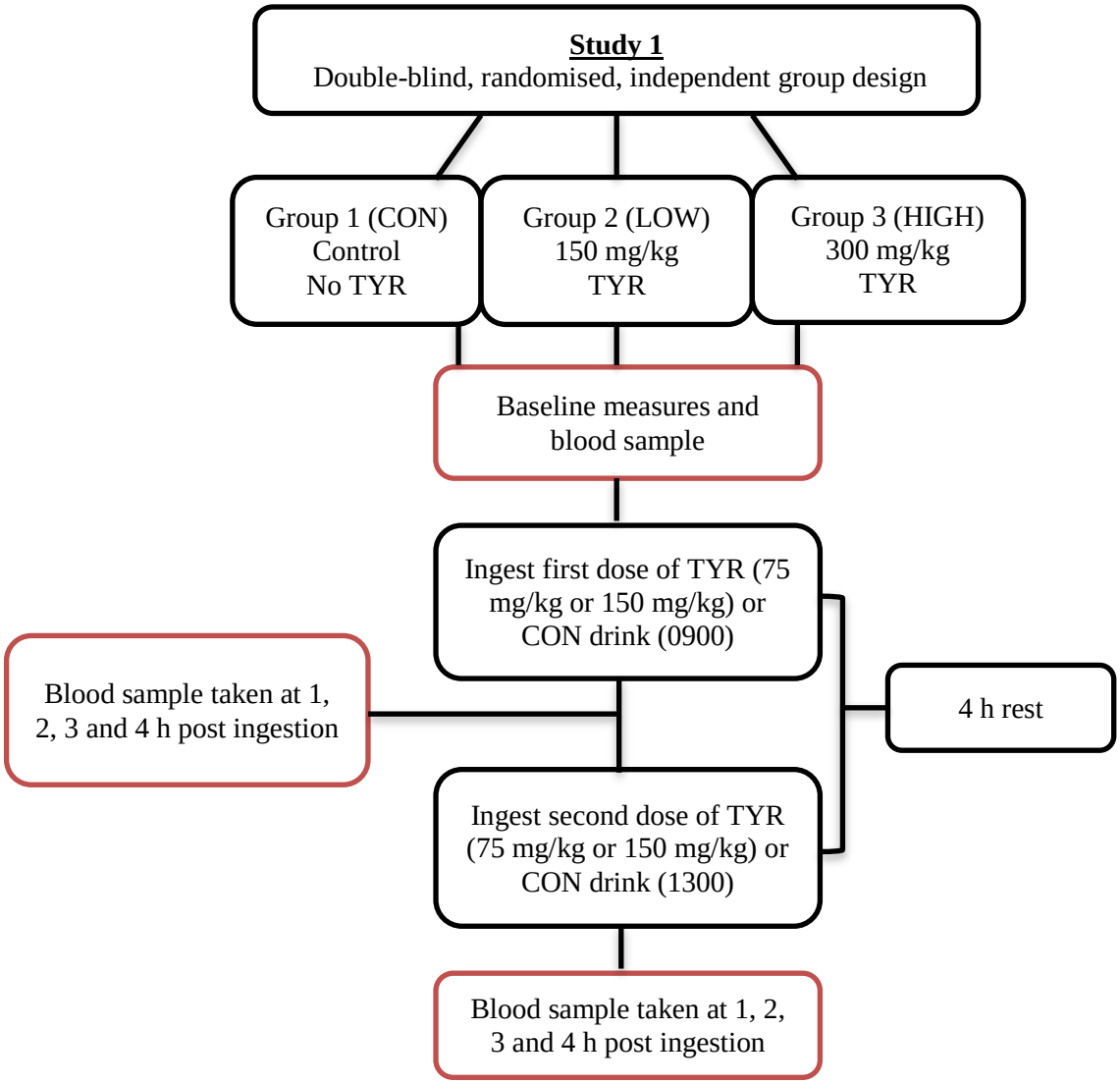

Figure 4.1. Schematic outlining the design and order of testing in study 1.

4.3.3 Standardised meals

Breakfast and 250 mL water was provided on arrival at the laboratory (0800) after an overnight fast. Standardised breakfast and lunch consisted of cornflakes (Kellogg's, UK) and semi-skimmed milk (Asda, Bedford, UK), which was weighed out (to nearest g and mL respectively) by the researcher for each participant, according to their resting energy expenditure. This was calculated using the following predictive metabolic equation by Mifflin *et al.* (1990):

$$10 \times \text{body mass (kg)} + 6.25 \times \text{height (cm)} - 5 \times \text{age (yrs)} - 161$$

The exact amount of cornflakes and milk equated to 20% of each participants total resting energy expenditure, which was consumed at both breakfast and lunch. Participants were instructed to consume this meal within 10 min to ensure standardisation between participants.

4.3.4 Blood collection

Frequent blood samples were required for this study, and therefore cannulation was the preferred method of blood sampling. A cannula (22G/1" Vasofix Safety, B Braun, UK) was inserted into an antecubital vein of the preferred arm and secured in place with a sterile dressing (Niko-Guard IV Dressing, DM Wood Medical, UK) to remain in place for a 9 h period (see Figure 4.2 for illustration). Blood samples were drawn from the inserted cannula at 9 time points (baseline, 1, 2, 3, 4, 5, 6, 7 and 8 h), directly into three separate Vacuette® containers (Vacuette® Grenier Bio-One, UK) treated with K_3 Ethylenediaminetetraacetic acid (EDTA) (1 x 5 mL tube) and Serum Clot Activator (2 x 6 mL tubes) in line with the Vacuette® recommendations (2007).

Figure 4.2. Illustration of blood drawn from inserted cannula.

4.3.4.1 K3EDTA Treated Blood (Plasma Volume)

Upon collection, EDTA treated blood was immediately pipetted from the K_3EDTA Vacuette® containers into heparinized capillary tubes (Hawksley & Sons Ltd, UK), centrifuged at 5000 rpm for 3 min (Hawksley Mircro Haematocrit centrifuge) and then measured from a Haematocrit reader (Hawksley, UK). Further blood from the same tube was pipetted into microcurvettes and analysed for haemoglobin concentration via a B-Haemoglobin photometer (Hb 201+, Hemocue Ltd, UK). Haematocrit and haemoglobin concentrations were measured in duplicate for each time point and the average of the two readings was recorded. Changes in plasma volume (PV) were then estimated from the values of haematocrit (Hct) and haemoglobin (Hb) using the following Dill and Costill (1974) method:

$$\% \Delta PV = [(Hb_{pre}/Hb_{post}) \times [(100 - Hct_{post})/(100 - Hct_{pre})] - 1] \times 100$$

$$\Delta PV = [(100 - Hb_b/Hb_a) - x\ 1 - (Hct_a - 100)/(1 - Hct_b - 100)] - 100$$

Where ΔPV is percentage change of PV, subscript b is baseline and subscript a is post. Blood results were subsequently adjusted as a result of changes in plasma volume.

4.3.4.2 Serum Clot Activator Treated Blood

The blood drawn into the Serum Clot Activator containers was left to clot at room temperature for 1 h before being centrifuged (ThermoScientific Multifuge, X3R) at 1500 g for 10 min at 4°C to yield serum in line with Watson *et al.* (2012) and the Vacuette® Handling Recommendations. The serum was then immediately removed from the clot, pipetted into a separate sterile container (CryoTube, Fisher Scientific, UK) and stored in duplicate at -80°C prior to analysis.

4.3.5 Amino Acid Analysis

Serum stored at -80°C for amino acid analysis was deproteinized using 12-M perchloric acid (70% PCA) (Sigma-Aldrich, UK) containing an internal standard (40 mM norvaline). Deproteinization was achieved by adding 12.5 µl of perchloric acid to an eppendorf tube with 500 µl serum. This was then thoroughly vortexed (Vortex Mixer, Phillip Harris) for 20 s before leaving on ice to cool for 15 min. Each tube was

centrifuged at high speed (10000-14000 rpm) and the resulting supernatant was stored on ice and neutralized with an equal volume of freon-trioctylamine (78% v/v 1,1,2-trichlorotrifluoroethane, 22% v/v trioctylamine). Amino acid concentrations of the filtered extract were determined by reverse-phase HPLC with ultraviolet detection using a Zorbax eclipse Amino Acid Analysis (AAA) column (4.6 × 75 mm, 3.5 μm) (Agilent Technologies UK Ltd, Cheshire, UK) with precolumn derivatization using orthophthalaldehyde (Henderson, Ricker, Bidlingmeyer, & Woodward, 2000). This method is in line with the amino acid analysis carried out by Watson *et al.* (2012).

Note: Amino acid analysis is for serum TYR concentration only. It was the authors aim to include LNAA data as well as the serum TYR data to highlight the ratio of TYR:LNAA in line with other previous research, however this data is unavailable to include in the thesis. This has been acknowledged as a limitation within the discussion of this chapter.

4.3.6 Experimental Procedure

Participants abstained from food or drink (other than water) after 2100 on the day prior to testing and until they arrived at the laboratory the next morning at 0800. Unless indicated otherwise, participants spent their time at rest within a temperature and humidity controlled laboratory and consumed meals in the adjoined food preparation room (both rooms were controlled at 19-21°C; 40-50% RH). Upon arrival at the laboratory, participants were immediately provided with breakfast, conforming to the standardised meal detailed in section 4.3.3. No additional food was consumed during the day other than the meals provided, however water was consumed ad libitum. Approximately 30 min post breakfast (~0830), a urine sample was provided by the participant and analysed to assess hydration status as described in section 3.1. Participants then completed a visual analogue scale (VAS) for gastric discomfort.

Immediately after the first blood sample (0900) after being seated for 30 min, participants consumed either the CON, LOW or HIGH TYR dose (see Figure 4.1 for schematic). The standardised meal was provided again for lunch (1200) and following this, the CON, LOW or HIGH TYR dose was again ingested. Total TYR ingested after both doses was 150 mg·kg body mass^{-1} in LOW and 300 mg·kg body mass^{-1} in

HIGH. This TYR dosing strategy (two separate doses of TYR, ingested 4 h apart) is in line with previous military (Mahoney *et al.*, 2007, O'Brien *et al.*, 2007) and sport specific (Coull *et al.*, 2015) based research. After the last blood draw at 1700, the cannula was removed and the participant was then free to leaving the laboratory.

4.3.7 Statistical analyses

An *a priori* power calculation (G*Power 3) was used to determine the number of participants required for this study (n = 7 in each group) with an alpha level of 0.05 and statistical power of 99%, using TYR concentrations reported in previous work (Watson *et al.*, 2012) and based on estimation (means = 50, 100 and 150, SD = 41) (Appendix G). Main statistical analyses were completed using linear mixed models (IBM SPSS statistics for Macintosh, Version 20, Armonk, NY) to assess mean differences in baseline anthropometric data, serum TYR concentrations, RTIPE and RTIME, gastric discomfort and blood pressure over time and between groups (CON, LOW and HIGH). Where significance was obtained, Sidak post-hoc tests were used to locate significant pairs. Ninety-five percent confidence intervals (95% CI) were also presented where necessary. Two-tailed statistical significance was accepted at the $p < 0.05$ level.

4.4 Results

4.4.1 Anthropometric data

No significant differences were observed in participants' baseline anthropometric data between the CON, LOW or HIGH group, including age ($F_{2,18}$ = 2.10, p = 0.15), height ($F_{2,18}$ = 1.03, p = 0.38), body mass ($F_{2,18}$ = 0.75, p = 0.49) and body fat percentage ($F_{2,18}$ = 0.23, p = 0.80) (see section 4.3.1 for descriptive Table 4.1).

4.4.2 Serum Tyrosine concentrations

Baseline serum TYR concentrations were not significantly different (p > 0.05) between the CON (64.3 ± 10.6 μmol/L), LOW (61.5 ± 17.8 μmol/L) and HIGH (65.9 ± 12.6 μmol/L) group. Over the 9 h period, a significant main effect of group was observed in mean TYR concentration ($F_{2,18}$ = 86.71, p < 0.001) between CON (60 ± 11.1 μmol/L), LOW (214.7 ± 72.9 μmol/L) and HIGH (309.8 ± 120.5 μmol/L). On average, TYR concentration in the HIGH group was 44% and 415% higher than the LOW (p < 0.001, 95% CI = 44.6-145.7 μmol/L) and CON (p < 0.001, 95% CI = 199.3-300.4 μmol/L) group, respectively (Figure 4.3). A significant effect of time ($F_{8,144}$ = 46.97, p < 0.001) and group x time interaction effect ($F_{16,144}$ = 14.74, p < 0.001) was also observed. The peak in serum TYR occurred 2 h post the second dose in both the HIGH and LOW group with the HIGH dose producing the largest peak (399.5 ± 69 μmol/L) compared to the LOW dose (278.5 ± 76.4 μmol/L) (p < 0.001, 95% CI = 55.5-186.5 μmol/L). Despite this, the second dose of TYR did not significantly elevate serum TYR concentrations from the first dose, in either the HIGH (350 vs 399 μmol/L respectively: p = 0.38, 95% CI = -113.75-14.6 μmol/L) or LOW (221 vs 279 μmol/L respectively: p = 0.13, 95% CI = -122.2-6.2 μmol/L).

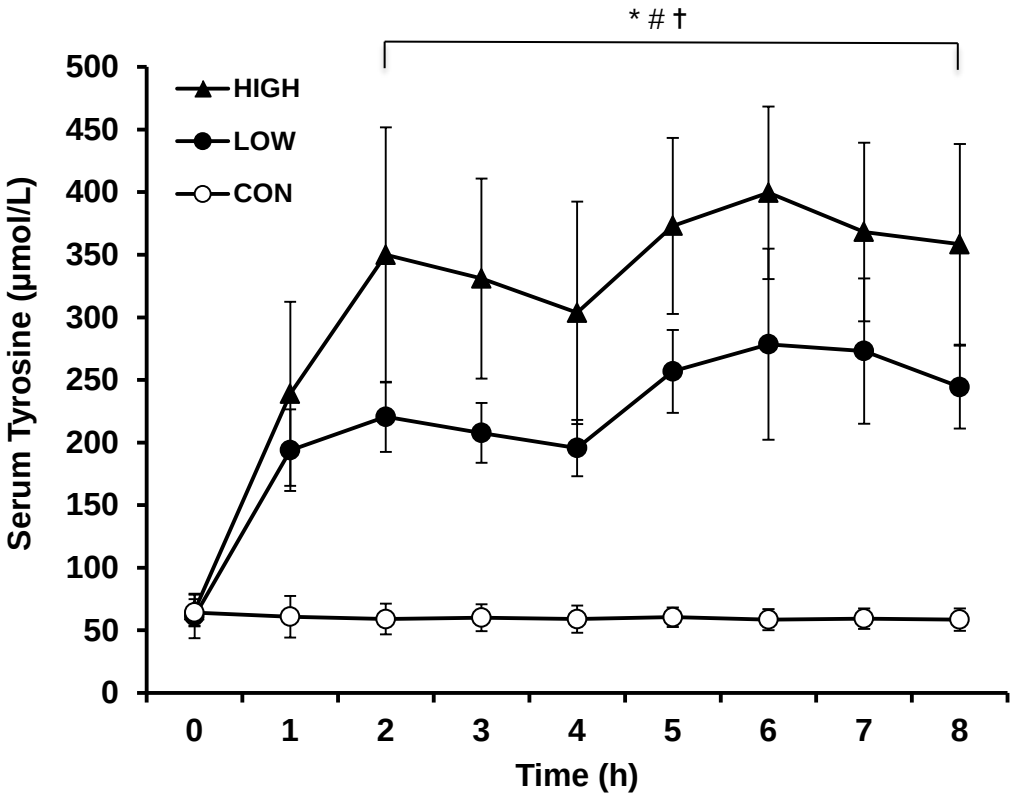

Figure 4.3. Mean serum TYR concentrations in each group (CON, LOW and HIGH), across all time points. Values are mean ± SD. *Denotes significant differences between conditions, #significant differences over time, †significant group x time interaction ($p < 0.05$).

4.4.4 Gastric discomfort

There was a significant effect of time for mean gastric discomfort ($F_{8,144} = 4.57$, $p < 0.001$) with highest ratings occurring at baseline which was after breakfast and before any supplement was consumed (21 ± 21 mm in CON, 39 ± 74 mm in LOW and 23 ± 45 mm in HIGH). No other significant differences were observed over time ($p > 0.05$) throughout the remainder of the testing day. Furthermore, no significant main effect of group was observed ($F_{2,18} = 0.36$, $p = 0.7$) between CON (5 ± 11 mm), LOW (11 ± 30 mm) and HIGH (3 ± 16 mm) and there was no significant group x time interaction effect ($F_{16,144} = 4.80$, $p = 0.95$) for mean gastric discomfort.

4.5 Discussion

The aim of this experimental chapter was to investigate the acute effects of different TYR doses (CON, LOW and HIGH) on serum TYR concentrations at rest. As expected, the main finding was that the HIGH dose (2 doses of 150 mg·kg body mass^{-1} TYR) elevated serum TYR concentrations significantly more than the LOW dose (2 doses of 75 mg·kg body mass^{-1} TYR) and CON (no TYR). Furthermore, it was observed that ingestion of a second, identical dose of TYR, 4 h post the first dose, did not significantly increase the peak in serum TYR in either the LOW or HIGH group. These findings suggest that ingestion of a single dose of 150 mg·kg body mass^{-1} TYR is sufficient to significantly elevate serum TYR concentrations (fivefold increase from baseline) and that a second dose, as administered in previous studies (Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Lieberman *et al.*, 2014, Coull *et al.*, 2015), may not provide any additional worth while increase.

To the author's knowledge, only one previous study has directly investigated the effects of acute oral TYR ingestion on amino acid concentrations at rest, in humans (Glaeser *et al.*, 1979). Although assessing smaller, single doses of TYR (100 and 150 mg·kg body mass^{-1}) the findings of Glaeser *et al.* (1979) support the present results, demonstrating dose-related increases with significantly higher elevations observed after ingestion of larger doses of TYR. Furthermore, peak elevations in blood TYR occurred 2 h post-ingestion for all dosages in the current study and in the investigation by Glaeser *et al.* (1979). Additionally, in the present study this 2 h post-ingestion peak also occurred after ingestion of the second dose of TYR.

The findings of this experimental chapter may be of use to researchers conducting future investigations involving TYR and also to athletes, military personnel and other individuals interested in the use of TYR for performance purposes. From the available data, individuals can identify the optimal time to consume TYR, depending on the nature of their sport or occupation, to ensure a sufficient elevation in blood TYR concentration occurs. The present study revealed that ingestion of a double dose of 150 mg·kg body mass^{-1} TYR did not significantly increase serum concentrations of TYR compared to a single dose. This may be beneficial in situations with limited time before exposure to a stressful stimulus, in which individuals may consume a single dose of 150 mg·kg body mass^{-1} TYR 1-2 h prior and still benefit from similar rises in

circulating TYR, instead of a prolonged double dose administration strategy. For example, prior to military operations in extreme conditions, a quick and simple administration strategy may be more appropriate and appealing to personnel. Moreover, as no side effects were observed in the present study and previous others, it can be suggested that TYR is a safe nutritional supplement when ingested acutely (Glaeser *et al.*, 1979, Sole *et al.*, 1985, Banderet and Lieberman, 1989, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Tumilty *et al.*, 2011, Coull *et al.*, 2015).

Although the findings of this study may be a useful guide, it is not certain that a significant rise in blood TYR concentration will translate to significant improvements in performance. However, it is known that increasing the availability of TYR through oral supplementation increases its ratio to other LNAA, which is the primary determinant of TYR uptake across the BBB and DA synthesis (Fernstrom and Faller, 1978). Furthermore, there is evidence to show that acute depletion of plasma TYR (via a TYR and phenylalanine free mixture), reduces the ratio of TYR:LNAA and consequently decreases exercise capacity in the heat (Tumilty *et al.*, 2014), highlighting the importance of TYR availability during acute stress. Therefore, it may be speculated that a fivefold increase in serum TYR observed in the present study after ingestion of 150 mg·kg body mass^{-1} TYR, might provide ergogenic benefits during extreme stress.

The methodology of experimental chapter 1 contains several limitations, which should be considered in future research. The use of only two different doses of TYR (150 and 300 mg·kg body mass^{-1}) limits the scope of the investigation. Assessment of a variety of doses and administration strategies may have provided better dose response relationship data, however as the majority of previous research utilises either 150 or 300 mg·kg body mass^{-1} TYR, it seemed appropriate to assess these two commonly utilised doses, which have had experimental effects within humans previously (Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Tumilty *et al.*, 2011, Coull *et al.*, 2015). It would have been ideal to assess the effects of 150 and 300 mg·kg body mass^{-1} TYR in single doses, but due to ethical considerations, it was not possible to assess such large doses of TYR without administering in two separate doses. Another limitation of the present chapter is the absence of LNAA data, which means that TYR cannot be expressed as a ratio with other competing amino acids. As this ratio is a

major factor in the transportation of TYR across the BBB not including this data limits the conclusions that can be made regarding the effects of elevating serum TYR. Furthermore, the lack of performance measures within this investigation may be considered a limitation. Inclusion of cognitive testing throughout the 9 h testing period may have provided useful information, however as TYR is suggested to exert its beneficial effects during acute stress, it is likely that there would have been no change in cognitive function between groups under the present conditions. Therefore, the sole purpose of experimental chapter 1 was to inform and identify a suitable dose to be assessed in experimental chapter 2 and in future research.

4.6 Conclusion

In conclusion, this study demonstrates that ingestion of a single dose of 150 mg·kg body mass^{-1} TYR may be sufficient to elevate serum TYR concentrations (350 μmol/L) without the need for a second dose. Future research should investigate the effects of 150 mg·kg body mass^{-1} TYR on performance and mood in variety of stressful environments.

CHAPTER 5: Experimental Chapter 2 - Effect of Tyrosine Ingestion on Cognitive Function and Load-carriage Performance in the Heat

5.1 Introduction

Military based operations and occupational pursuits are often performed in extreme environments, commonly in hot and/or humid conditions (Marriott, 1993, Hocking *et al.*, 2001). Deployment overseas in countries with semi-arid environments (such as Iraq or Afghanistan) may result in prolonged exposure to high ambient temperatures ≥40°C. During such extreme heat exposure, military personnel may be required to carry out demanding physical and cognitive tasks whereby the outcome could be life threatening, thus optimal performance is paramount (Orasanu and Backer, 1996). It is well established that prolonged exercise-heat-stress impairs both exercise performance and cognitive function, attributed to both peripheral and central factors, whereby fatigue occurs prematurely (Cheung and Sleivert, 2004, Nybo *et al.*, 2014). Furthermore, this thermal strain may be exacerbated when wearing protective clothing and carrying heavy loads (Havenith, 1999, Cheung *et al.*, 2000, Dewhurst *et al.*, 2014), therefore the military are frequently investigating ways to alleviate such impairments for safety, protection and performance purposes (Nindl *et al.*, 2002).

Nutritional manipulation strategies are commonly used to dampen the adverse affects associated with heat-stress, through various centrally mediated pathways (Lieberman, 2003, Roelands and Meeusen, 2010, Meeusen, 2014). The amino acid TYR exerts its effects by increasing catecholamine synthesis, which is suggested to improve motivation, arousal and motor control during stress (when catecholamine concentration begins to deplete) (McMorris *et al.*, 2006, Watson, 2008, Kishore *et al.*, 2013). As previously discussed, the use of TYR is relatively popular in the US military however, recent evidence from controlled laboratory settings, question its efficacy (Watson *et al.*, 2012, Tumilty *et al.*, 2014), and thus further research is required to elucidate this.

In experimental chapter 1, the pharmacokinetics of two separate doses of TYR were examined and it was established that a single dose of 150 mg·kg body mass^{-1} TYR was sufficient to elevate serum TYR levels at rest, without inducing any side effects. It is

yet to be investigated whether this dose of TYR may influence exercise performance or cognitive function in a laboratory controlled, simulated military setting, in a hot environment. Moreover, the only previous studies assessing the effects of TYR in extreme heat (>30°C) were either conducted on rodents (Lieberman *et al.*, 2005) or using passive heat protocols (Kishore *et al.*, 2013), thus there is a need to investigate this area further.

5.2 Aim and Hypotheses

The purpose of this study was to utilise the identified dose of TYR (150 mg·kg body mass^{-1}) from the findings of experimental chapter 1 and investigate its effects on physical and cognitive performance utilising a military based protocol (25 kg load-carriage) in the heat (40°C; 30% RH). It was primarily hypothesised that ingestion of 150 mg·kg body mass^{-1} TYR would significantly improve aspects of cognitive function compared to placebo. Secondly, it was hypothesised that TYR would not influence 2.4 km time-trial performance.

5.3 Methodology

5.3.1 Participants

Eight healthy, recreationally active, male volunteers participated in this study (mean ± SD: age 23 ± 1 y, height 176.4 ± 5.9 cm, body mass 79 ± 11.5 kg, body fat percentage 13.1 ± 2.8%, $\dot{V}O_{2max}$ 53.4 ± 5.7 $ml \cdot kg \cdot min^{-1}$ and activity level 7.1 ± 1.8 $h \cdot wk^{-1}$). According to recent guidelines to classify subject groups (albeit cycling based), the participants in the present study fall under performance level 2, which indicates that they are recreationally trained (De Pauw *et al.*, 2013). Inclusion and exclusion criteria is detailed in section 3.1

5.3.2 Experimental Design

This study employed a double-blind, counter-balanced, crossover design in which participants visited the laboratory on four separate occasions; two familiarisation sessions and two experimental conditions. During the experimental conditions, participants either ingested TYR and or a placebo (see Figure 5.1 for study overview schematic). All visits to the laboratory were separated by at least 7 d to allow for a full recovery and all main experimental trials were performed at the same time of day (± 30 min) to minimise the influence of circadian variation (Drust *et al.*, 2005).

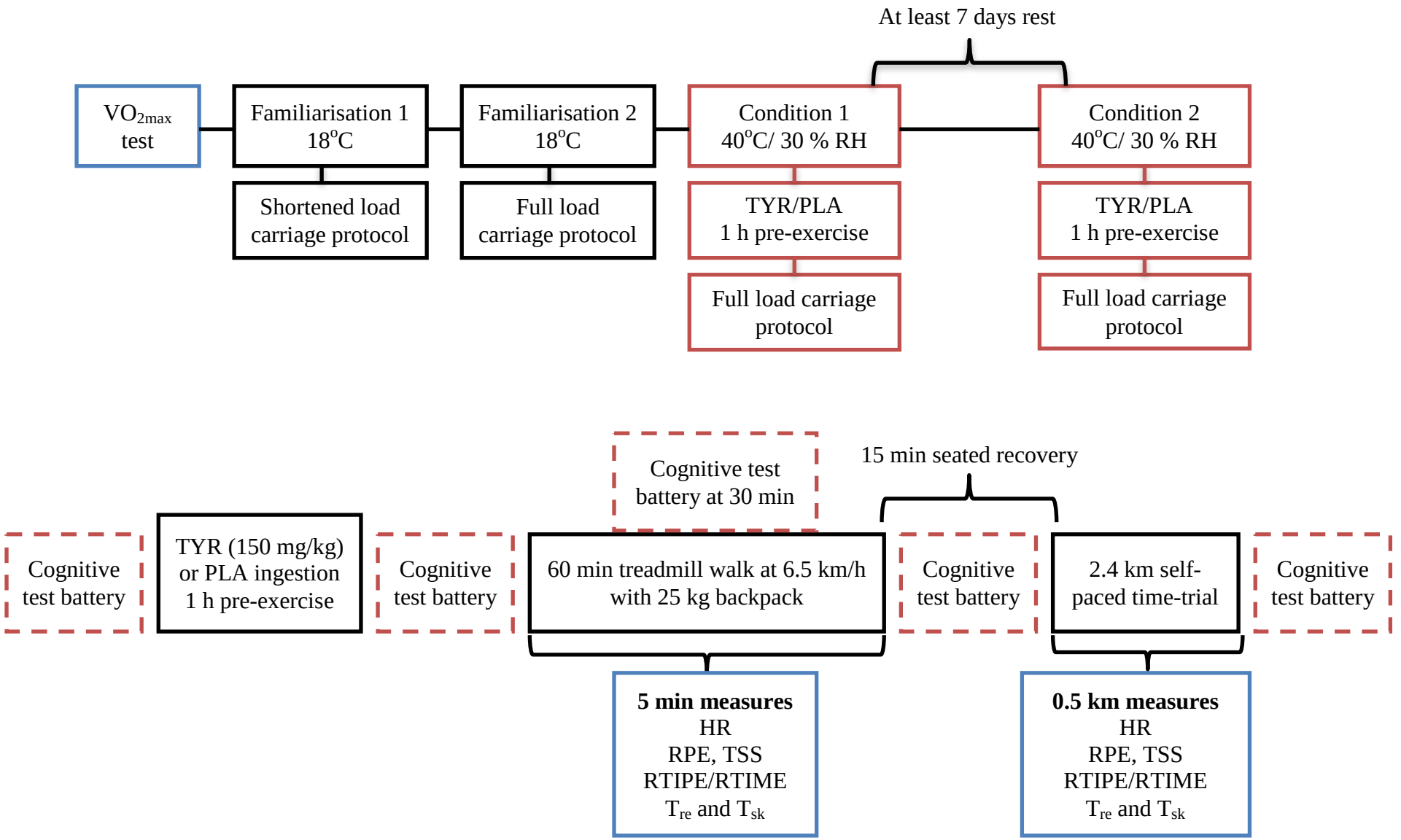

Figure 5.1. Schematics detailing an overview and the experimental procedures of study 2 (double-blind, counterbalanced, crossover design) All main experimental trials were performed in 40°C; 30 % RH.

5.3.3 Familiarisation

All participants were required to attend two familiarisation sessions prior to the experimental conditions to ensure there was no learning effect or systematic bias within the main trials. During the first session participants were familiarised and fitted with a 25 kg weighted backpack (Infantry Bergen, Millar-tree, Bedford, UK) and subsequently completed 20 min exercise (6.5 $km.h^{-1}$, 0% gradient) on a motorised treadmill (Woodway, PPS55 Med-I, Cranlea, UK), in temperate conditions (18°C; 40% RH). The familiarisation sessions were not performed in hot conditions to avoid heat acclimation from repeated heat exposure in line with Coull *et al.* (2015). After 15 min of seated recovery, participants then completed a 2.4 km self-paced time-trial carrying the 25 kg load on the same treadmill. The familiarisation employed in the present study was deemed appropriate to minimise any learning effects and is in line with Faghy and Brown (2014a) (load-carriage protocol) and Hope *et al.* (1998), Coull *et al.* (2015), Taylor *et al.* (2014), Watkins *et al.* (2014) (cognitive testing).

The backpack load, walking speed and time-trial distance are in line with previous work on load-carriage performance, specifically related to military and occupational settings (Blacker *et al.*, 2011, Faghy and Brown, 2014a, Faghy and Brown, 2014b). This specific protocol was designed and validated to closely reflect the physiological characteristics encountered in the military, enabling the assessment of deployment readiness and responses to relevant interventions (Faghy and Brown, 2014a). This protocol has shown good reproducibility as a measurement of load-carriage performance (interclass correlation (ICC): 0.85 and coefficient of variation (CV): 10.53%) compared to the recommended level of acceptable error (Currell and Jeukendrup, 2008, Brughelli and Van Leemputte, 2013, Faghy and Brown, 2014a). The mass of the backpack load (25 kg) was equally distributed and worn in accordance with the manufacturer's guidelines. The backpack was positioned individually to suit each participant by adjusting the shoulder and waist straps and this was recorded to the nearest mm to remain the same throughout both experimental trials. The second session involved full familiarisation with the protocol as described below in section 5.3.5, but in temperate conditions. Demonstration versions of the cognitive tests (described in detail in section 5.3.5) were performed before, during and after exercise in both familiarisation sessions.

5.3.4 Pre-Experimental Procedures

Prior to the commencement of the main experimental trials, all participants completed a maximal oxygen consumption ($\dot{V}O_{2max}$) assessment on a motorised treadmill (Woodway, PPS55 Med-I, Cranlea, UK) using a standardised incremental protocol (ACSM, 2013). Participants were fitted with a heart rate (HR) monitor and suitably sized metalyser mask (Cortex, Cranlea, UK) and after a 5 min self-paced warm up, exercised until volitional exhaustion. Online breath-by-breath analysis (Cortex, Metalyser 3B, Cranlea) was used to determine $\dot{V}O_{2max}$ after full calibration of the online system. Measures of HR and rating of perceived exertion (RPE) were recorded every minute for use as secondary criteria. For more details on other pre-experimental procedures and controls refer back to section 3.1.

5.3.5 Experimental Procedures

Participants attended the laboratory on two separate occasions for the two main trials (TYR and placebo) (Figure 5.1 for study schematic). See section 3.1 for details on experimental controls. Upon arrival at the laboratory, participants rested for 5 min in a seated position before completing the first cognitive test battery which included a vigilance, dual-task and simple reaction time test utilising the psychomotor evaluation software (PsychE) (Hope *et al.*, 1998). The cognitive tests were all performed on a laptop and have previously been utilised to assess the effects of extreme environmental conditions on decision-making (Taylor *et al.*, 2014, Watkins *et al.*, 2014) and the effects of TYR ingestion in the heat (Coull *et al.*, 2015).

The vigilance tests (2 min duration) required participants to focus on a laptop screen in which three-digit numbers appeared at a rate of 100 per min with an 8% duplication rate (Figure 5.2). Participants had to identify when the same number appeared twice in row by pressing the spacebar and were assessed on the amount of HIT (correct response), MISS (missed cue) and FALSE (false response) scores they achieved. The dual-task test (3 min duration) required participants to track a moving target with the mouse cursor and simultaneously respond to random stimuli (Figure 5.3). The percentage of time successfully on target (TRACKING) and stimuli responses (MISS and FALSE) were recorded (see Table 5.1 for descriptions of the vigilance and dual-task cognitive tests). The third test measured reaction time and involved participants

holding down the spacebar until a random object appeared on the screen (between 1-10 s), to which they reacted by releasing the spacebar and pressing a target key (numeric key 4-9) with the same finger (Figure 5.4). The test consisted of 20 stimuli presentations and for each response, thinking time (THINK) and movement time (MOVE) were recorded to an accuracy of 1 ms.

All cognitive tests were laptop based and were employed in line with the descriptions provided by Hope *et al.* (1998). The tests were employed to enable a direct comparison with previous work from our research group, utilising the same cognitive test software (Coull *et al.*, 2015) and for ease of administration and relevance to the military. For reliability, pre-supplement cognitive test values (vigilance, dual-task and simple reaction time) in TYR and PLA were compared to generate reliability statistics, which are reported within the results section. See statistical analysis for calculation methods and Table 5.2 for reliability statistics.

Table 5.1. Descriptions of each cognitive test response (HIT, MISS, FALSE and TRACKING) for vigilance and dual-task assessments (Coull *et al.*, 2015).

Response	**Vigilance**	**Dual-task**
HIT	Correctly identifying a duplicate number	N/A
MISS	Failing to identify a duplicate number	Failing to identify a present icon
FALSE	Incorrectly identifying a duplicate number	Incorrectly identifying an icon is present when it is not
TRACKING	N/A	Ability to track a moving target (%)

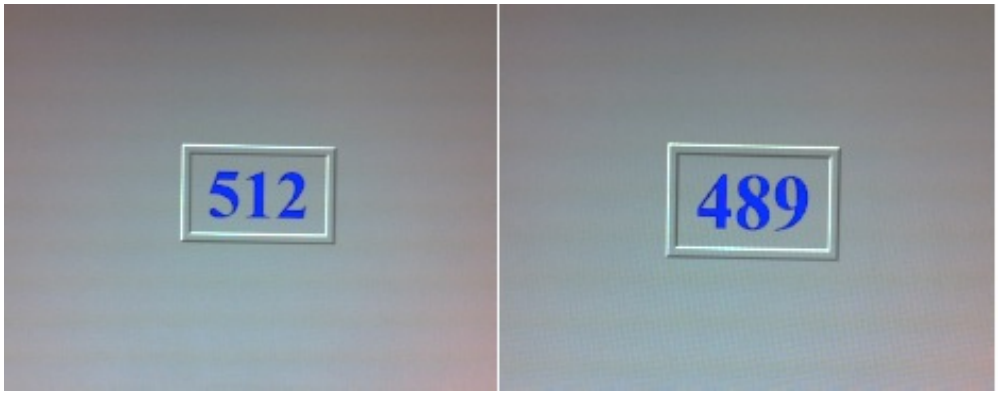

Figure 5.2. Screenshots of the PsychE software vigilance test.

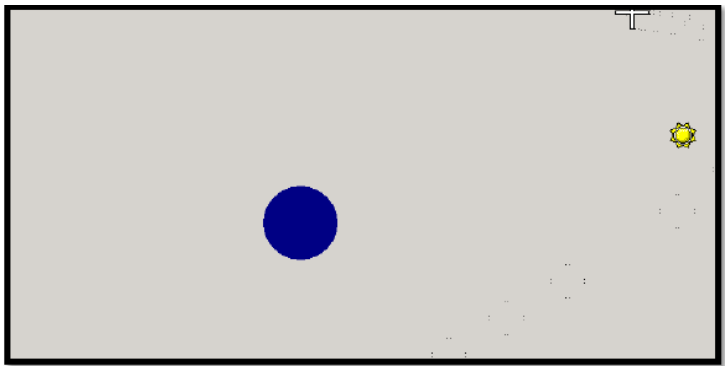

Figure 5.3. Screenshot of the PsychE software dual-task test being completed

Figure 5.4. Image of the PsychE software simple reaction test being completed.

After the baseline cognitive test battery, participants then orally ingested a placebo [PLA (250 mL sugar free lemon squash) (Tesco, UK)] or TYR (same as PLA plus 150 mg·kg body mass^{-1} TYR powder) (Nutricia Ltd, UK). They were instructed to consume this within 5 min, and then rest within the laboratory for 1 h before commencing the protocol. Participants were fitted with a HR monitor (Polar, FS1, Cranlea), rectal thermometer (Henleys, 400H and 4491H) inserted 10 cm past the anal sphincter, and skin thermistors (Grant, EUS-U-VS5-0, Wessex Power) attached to four skins sites; upper arm, chest, thigh and calf (Ramanathan, 1964). Subsequently, the following resting measures were recorded; HR, RPE [6-20 Borg scale (Borg, 1982)], thermal sensation (TSS) [0-8 scale (Young *et al.*, 1987)], readiness to invest mental (RTIME) and physical effort (RTIPE), core (T_{re}) and skin (T_{sk}) temperature and pre-exercise body mass (Digital Tanita Weighing scales, BWB0800, Allied Weighing, UK). The RTIME/RTIPE were measured on VAS and were used for a comparison between the current and a previous study (Coull *et al.*, 2015).

Approximately 1 h post TYR ingestion, participants then completed another cognitive test battery before entering the environmental chamber (T.I.S.S Services UK, Hampshire, UK), set at 40°C; 30% RH. Once inside the chamber, participants were fitted with the 25 kg backpack before commencing the 60 min load-carriage protocol, in which they were required to walk at a speed of 6.5 km.h^{-1} with a 0% incline (Faghy and Brown, 2014a). Halfway through the 60 min walk (at 30 min), participants completed further cognitive assessments (vigilance and simple reaction time only), while walking on the treadmill, via a custom-built laptop stand (see Figure 5.5).

Figure 5.5. Simulated military walk in the environmental chamber (a) and participant completing cognitive tests on a treadmill with custom built stand during familiarisation (b).

The 60 min walk was followed by 15 min seated recovery, in which participants removed the backpack and completed further cognitive assessments within the environmental chamber. During the recovery period in the first trial (either PLA or TYR), participants were provided with 1 L of water after completing the cognitive assessments and were instructed to drink this ad libitum. The total amount of water consumed was recorded and the same amount was provided in the second trial (either PLA or TYR), to ensure fluid intake was standardised. Participants then commenced a 2.4 km time-trial with the 25 kg loaded backpack, which they were instructed to complete as fast as possible (Faghy and Brown, 2014a). On completion of the time-trial, participants completed the final cognitive test battery before evacuating the chamber into a thermo-neutral room. Subsequently, a post-exercise body mass recording was obtained, and finally participants were provided with a drink and asked to remain seated in the thermo-neutral room until T_{re} returned to baseline.

5.3.5.1 Measurements

During the load-carriage protocol, physiological (HR), perceptual (RPE and TSS) and temperature (T_{re} and T_{sk}) measures were recorded at 10 min intervals. Specific data loggers were used to record T_{re} (Measurement Specialties, 4600, Henleys Medical) and T_{sk} (Grant, Squirrel Series, model 451, Wessex Power) and weighted mean T_{sk} was calculated from the temperatures recorded for all four skin sites using the Ramanathan (1964) equation where '*t*' represents temperature:

$$0.3(t_{chest} + t_{arm}) + 0.2(t_{thigh} + t_{leg})$$

Additionally, at 15 min intervals, RTIPE/RTIME and the temperature and humidity of the chamber were also recorded. During the entire protocol (60 min walk and 2.4 km time-trial) participants were unaware of the time elapsed and were only able to view the distance covered during the time-trial. Throughout the time-trial, participants communicated when they had reached certain distances (0.5, 1, 1.5, 2 and 2.4 km) to enable the researcher to record the physiological, perceptual and temperature measurements. In total, cognitive function was measured at five separate time points; pre-supplementation, pre-exercise, during exercise (30 min into load carriage protocol while walking on the treadmill), during the 15 min break and post time-trial. However, the dual-task test was not performed during exercise due to the difficulty

using a mouse while walking on the treadmill. Physical performance was assessed using the 2.4 km time-trial times recorded in both TYR and PLA conditions. Sweat loss was calculated from the difference in pre- and post-exercise body mass, after adjusting for fluid intake and urine loss (during the 15 min break).

Note: Venous blood samples were taken in this study to assess serum TYR concentrations and LNAA ratio using standardise venepuncture technique (pre-supplementation, pre-exercise and immediately after the last cognitive assessment). Analyses of these samples were due to be carried out at another institution in line with the amino acid analysis in experimental chapter 1, however due to time and specific other restraints this was not possible and thus data is not available to present within this thesis.

5.3.6 Statistical analysis

An *a priori* power calculation (G*Power 3) was used to determine the number of participants required for this study (n = 8) with an alpha level of 0.05 and statistical power of 99%, using data (time-trial completion times) from previous load-carriage research (Faghy and Brown, 2014a). Statistical analysis was completed using linear mixed models (IBM SPSS statistics for Macintosh, Version 20, Armonk, NY) to analyse mean differences in all variables between the two conditions (TYR and PLA). Where significance was obtained, Sidak post-hoc tests were used to locate significant pairs. Ninety-five percent confidence intervals (95% CI) were also presented where necessary. Two-tailed statistical significance was accepted at the $p < 0.05$ level. The reproducibility of the cognitive function tests (pre-supplementation baseline tests; n = 8) was calculated using the change in mean, coefficient of variation (CV), intraclass correlation coefficient (ICC) and typical error (TE) utilising Microsoft Excel.

5.4 Results

5.4.1 Cognitive function

Linear mixed model analysis revealed that there was no systematic bias for the cognitive test measures. Reproducibility statistics for vigilance, dual-task and reaction time are reported in Table 5.2 (pre-supplementation resting values in TYR and PLA). Vigilance MISS and FALSE and dual-task FALSE statistics (ICC, CV and typical error (TE)) were found to be unreliable from the available data.

Table 5.2. Reliability data for cognitive tests measured at rest.

Variable	*P* value	ICC	CV (%)	TE (%)
Vigilance: HIT	0.90	0.87	13.90	0.48
Vigilance: MISS	0.90	0.76	64.70	0.67
Vigilance: FALSE	0.73	0.53	55.10	1.10
Dual-task: TRACKING	0.94	0.50	7.80	1.15
Dual-task: MISS	0.72	0.70	10.90	0.25
Dual-task: FALSE	0.72	0.32	40.40	0.63
Reaction time: THINK	0.60	0.80	10.30	0.67
Reaction time: MOVE	0.67	0.80	14.40	0.60

5.4.1.1 Vigilance

There was no significant main effect for condition for vigilance HIT scores between TYR and PLA ($F_{1,63}$ = 0.22, p = 0.64). There was also no significant effect of time ($F_{4,63}$ = 2.21, p = 0.08) or condition x time interaction ($F_{4,63}$ = 1.89, p = 0.12). Similarly, there was no significant main effect for vigilance MISS scores ($F_{1,63}$ = 0.09, p = 0.77) and no significant effect of time ($F_{4,63}$ = 1.81, p = 0.14) or condition x time interaction ($F_{4,63}$ = 1.96, p = 0.11). There was a significant effect of time for vigilance FALSE scores ($F_{4,63}$ = 5.79, p < 0.001) with higher scores observed during the exercise bout (85% higher in TYR and 157% higher in PLA), compared to pre-exercise scores (p = 0.01, 95% CI = -2.7- -2.1). However, there was no effect of condition ($F_{1,63}$ = 0.79, p = 0.38) or condition x time ($F_{4,63}$ = 1.01, p = 0.41).

5.4.1.2 Dual-task

There was a significant effect of time for dual-task TRACKING scores ($F_{3,49}$ = 8.34, p < 0.001). On average TRACKING accuracy (%) was significantly lower after the 60 min bout of exercise (18% lower in TYR and 10% lower in PLA), compared to pre-exercise scores (p = 0.01, 95% CI = 4.7-22.7%). However, there was no significant main effect of condition ($F_{1,49}$ = 1.59, p = 0.21) or condition x time interaction effect ($F_{3,49}$ = 0.91, p = 0.44). No significant main effect of condition was observed for dual-task MISS scores ($F_{1,49}$ = 2.10, p = 0.16) and there was similarly no effect of time ($F_{3,49}$ = 1.46, p = 0.24) or condition x time interaction ($F_{3,49}$ = 2.15, p = 0.11). There was a significant effect of time ($F_{3,49}$ = 6.68, p < 0.01) and a significant condition x time interaction effect ($F_{3,49}$ = 6.58, p < 0.01) for dual-task FALSE scores but no significant main effect for condition ($F_{1,49}$ = 2.66, p = 0.11). On average FALSE scores increased after exercise in the heat and specifically after the 60 min exercise bout, FALSE scores were significantly higher in TYR compared to PLA (p < 0.01, 95% CI = 2-5).

5.4.1.3 Simple reaction time

A significant effect of time was observed for THINK time in the simple reaction test ($F_{4,63}$ = 3.62, p < 0.01). On average THINK time was slowest during exercise with a 19% increase in TYR and 29% increase in PLA from pre-exercise scores (p = 0.01, 95% CI = 16.9-161.9 ms). However, there was no significant main effect of condition ($F_{1,63}$ = 0.004, p = 0.95) or a significant condition x time interaction effect ($F_{4,63}$ = 0.30, p = 0.88). Similarly, there was a significant effect of time for MOVE time in the simple reaction test ($F_{4,63}$ = 4.96, p < 0.01), but no significant main effect for condition ($F_{1,63}$ = 0.07, p = 0.79) or condition x time interaction effect ($F_{4,63}$ = 0.24, p = 0.92). On average MOVE time was slower immediately post time-trial (26% slower in TYR and 26% slower in PLA), compared to pre-exercise (p = 0.048, 95% CI = 0.18-81.3 ms).

Table 5.3. Cognitive test scores in TYR and PLA conditions for all time-points measured. Values are mean ± SD. *Significant difference between conditions and #over time ($p < 0.05$).

		Cognitive test score				
Response	**Condition**	Pre-ingestion	Pre-exercise	30 min	60 min	Post TT
Vigilance	TYR	10.6 ± 3.6	10.1 ± 3.7	11.0 ± 4.0	9.4 ± 4.2	11.6 ± 3.2
HIT	PLA	10.5 ± 3	10.1 ± 3.2	8.8 ± 4.1	10.6 ± 3.2	11.6 ± 2.8
Vigilance	TYR	4.4 ± 2.8	5.3 ± 3.2	4.0 ± 3.7	5.8 ± 3.8	3.4 ± 2.9
MISS	PLA	4.5 ± 2.8	4.8 ± 3.2	6.1 ± 3.9	4.3 ± 3.2	3.8 ± 2.7
Vigilance	TYR	1.3 ± 0.9	1.8 ± 1.7	2.4 ± 2.1#	1.4 ± 1.3	1.0 ± 1.8
FALSE	PLA	1.4 ± 1.2	1.3 ± 1.0	3.6 ± 1.7#	1.5 ± 0.8	1.3 ± 0.7
Dual-task	TYR	67.6 ± 6.5	66.8 ± 6.6	No test	48.9 ± 19.3#	58.5 ± 19.5
TRACKING	PLA	67.3 ± 6.8	67.9 ± 5.4	No test	58.4 ± 13.5#	60.0 ± 12.0
Dual-task	TYR	1.1 ± 0.4	1.3 ± 0.5	No test	2.1 ± 1.4	1.3 ± 1.2
MISS	PLA	1.3 ± 0.5	1.1 ± 0.4	No test	1.1 ± 0.4	1.3 ± 2.4
Dual-task	TYR	0.5 ± 0.8	0.4 ± 0.5	No test	3.8 ± 2.8*#	1.3 ± 2.4#
FALSE	PLA	0.3 ± 0.7	0.6 ± 0.7	No test	0.6 ± 0.7#	2.1 ± 1.9#
Simple reaction	TYR	428.6 ± 68.6	402.9 ± 55.6	480.3 ± 114.6#	447.8 ± 128.2	429.0 ± 102.4
THINK (ms)	PLA	411.4 ± 69.0	391.7 ± 46.8	505.6 ± 192.0#	449.7 ± 104.1	456.6 ± 84.7
Simple reaction	TYR	145.1 ± 53.4	155.5 ± 59.8	186.1 ± 48.0	198.4 ± 76.6#	196.1 ± 75.1#
MOVE (ms)	PLA	153.5 ± 52.5	156.4 ± 49.9	168.8 ± 53.2	193.6 ± 75.1#	197.3 ± 67.0#

5.4.2 Time-trial performance

No significant difference was observed in time-trial completion time ($F_{1,14} = 547.9$, $p = 0.74$) between the TYR (19.78 ± 3.44 min) and PLA (20.29 ± 3.55 min) condition (Figure 5.6).

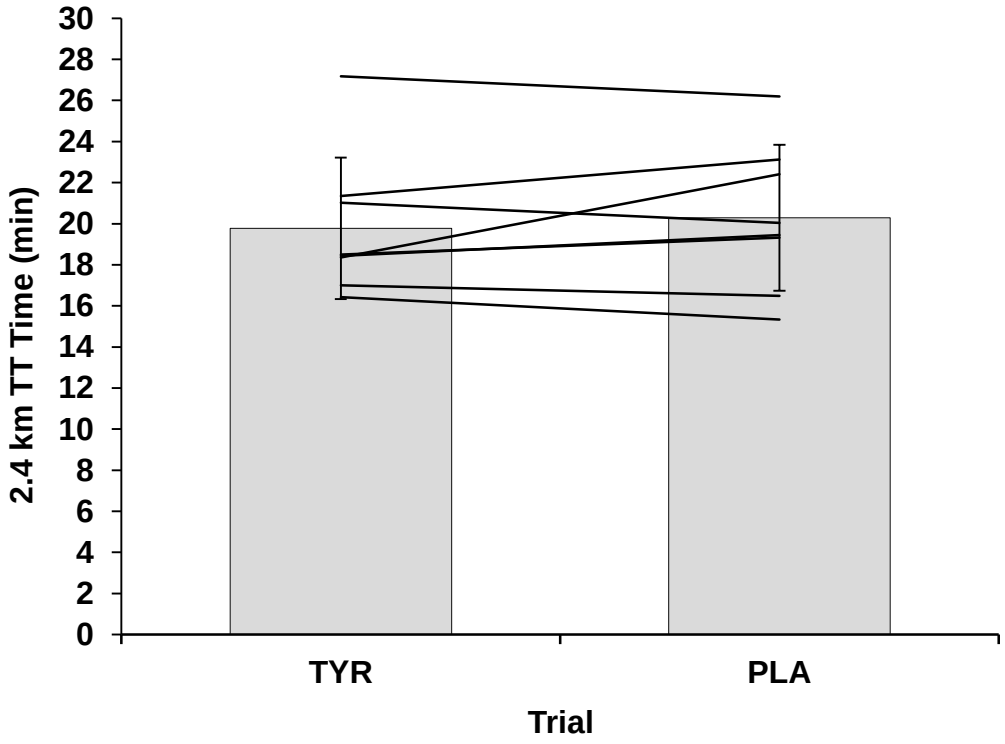

Figure 5.6. Group mean time-trial completion times (min) in both TYR and PLA conditions. Values are mean ± SD.

5.4.3 Heart rate

A significant effect of time ($F_{13,189} = 159.16$, $p < 0.001$) was observed for mean HR, with a mean increase of 127 b min^{-1} and 126 b min^{-1} from pre-exercise to immediately post the time-trial in TYR and PLA, respectively ($p < 0.001$, 95% CI = 93.4-159.9 b min^{-1}) (Figure 5.7). However, there was no significant main effect of condition for mean HR ($F_{1,189} = 5.84$, $p = 0.06$) between TYR (156 ± 14 b min^{-1}) and PLA (160 ± 15 b min^{-1}) and no significant condition x time interaction effect ($F_{13,189} = 1.59$, $p = 0.18$).

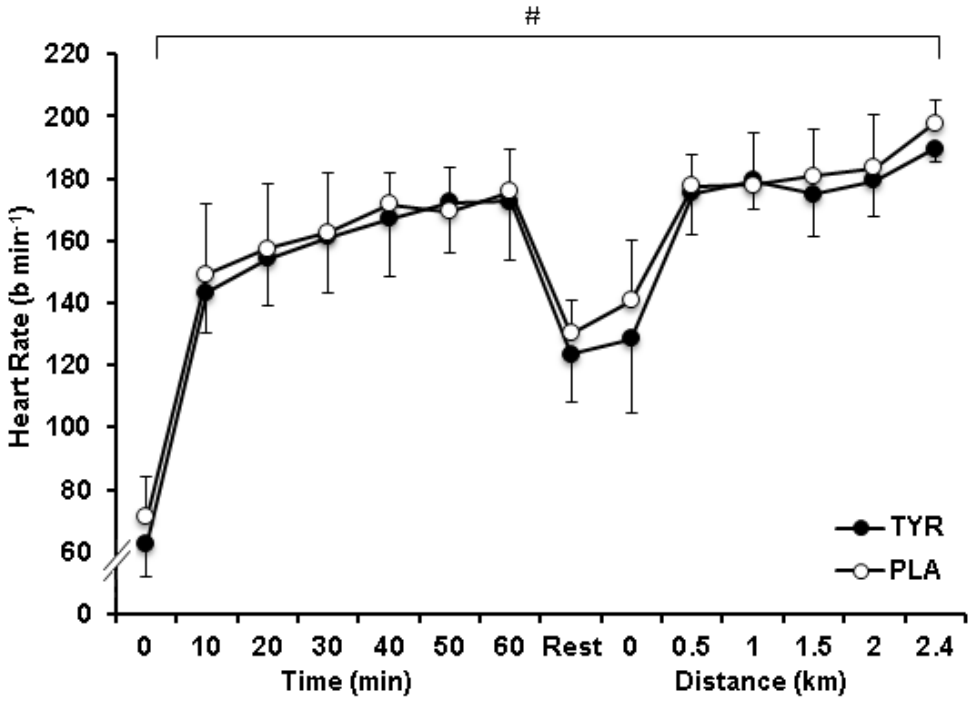

Figure 5.7 Group mean heart rate (b min^{-1}) responses in TYR and PLA during the 60 min walk, rest period and 2.4 km time-trial. Values are mean ± SD. #Significant differences over time ($p < 0.05$).

5.4.4 Temperature measures

There was a significant effect of time for mean T_{sk} ($F_{13,189} = 96.16$, $p < 0.001$), with increases during both bouts of exercise and a decrease during the rest period (Figure 5.8a). Highest mean T_{sk} values were observed at the end of the 60 min exercise (36.8 ± 0.9°C in TYR and 37.0 ± 0.5°C in PLA). No significant main effect of condition was observed for mean T_{sk} ($F_{1,189} = 3.1$, $p = 0.11$) between TYR (35.7 ± 0.9°C) and PLA (36 ± 0.7°C) and there was no condition x time interaction effect ($F_{13,189} = 0.19$, $p = 0.99$). Similarly, there was a significant effect of time for mean T_{re} ($F_{13,189} = 90.16$, $p < 0.001$), with an increase during both bouts of exercise and a decrease during the rest period (Figure 5.8b). Peaks in T_{re} were observed both at the end of the 60 min exercise bout and immediately post time-trial, however the highest T_{re} values were observed at then end of the time-trial (38.8 ± 0.4°C in TYR and 38.8 ± 0.5°C in PLA). No significant main effect for condition was observed for mean T_{re} ($F_{1,189} = 1.43$, $p = 0.23$) between TYR (38.2 ± 0.4°C) and PLA (38.2 ± 0.4°C) and there was no condition x time interaction effect ($F_{13,189} = 0.41$, $p = 0.97$).

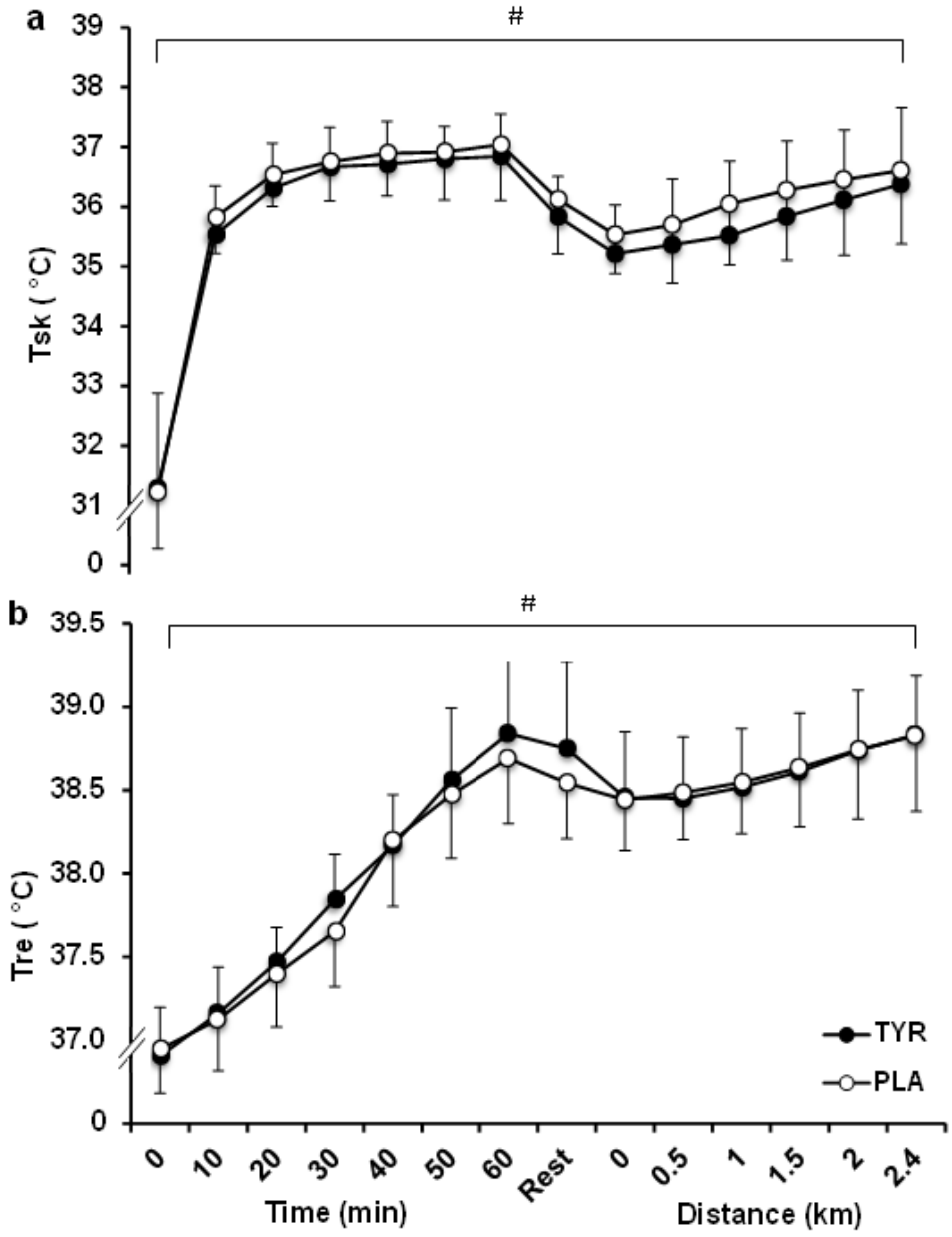

Figure 5.8 Group mean weighted skin temperature (a) and mean core temperature (b) responses in TYR and PLA during the 60 min walk, rest period and 2.4 km time-trial. Values are mean ± SD. #Significant differences over time ($p < 0.05$).

5.4.5 Subjective measures

A significant effect of time was observed for mean TSS ($F_{13,189} = 93.1$, $p < 0.001$), with an increase throughout both bouts of exercise, reaching end values of 7 ± 1 in TYR and 7 ± 1 in PLA, indicating that participants felt 'very hot' to 'unbearably hot' (Figure 5.9a). No significant main effect for condition was observed in mean TSS scores ($F_{1,189} = 1.35$, $p = 0.25$) between TYR (6 ± 1) and PLA (6 ± 1) and there was no significant condition x time interaction effect ($F_{13,189} = 0.62$, $p = 0.84$). Similarly,

there was a significant effect of time for mean RPE ($F_{13,189}$ = 131.61, p < 0.001) as RPE values increased throughout both bouts of exercise but were highest on completion of the time-trial, reaching values of 19 on the Borg scale in both TYR and PLA (Figure 5.9b). There was no significant main effect of condition for mean RPE scores ($F_{1,189}$ = 1.22, p = 0.27) between TYR (15 ± 2) and PLA (15 ± 1) and no significant condition x time interaction effect was observed ($F_{13,189}$ = 0.96, p = 0.49).

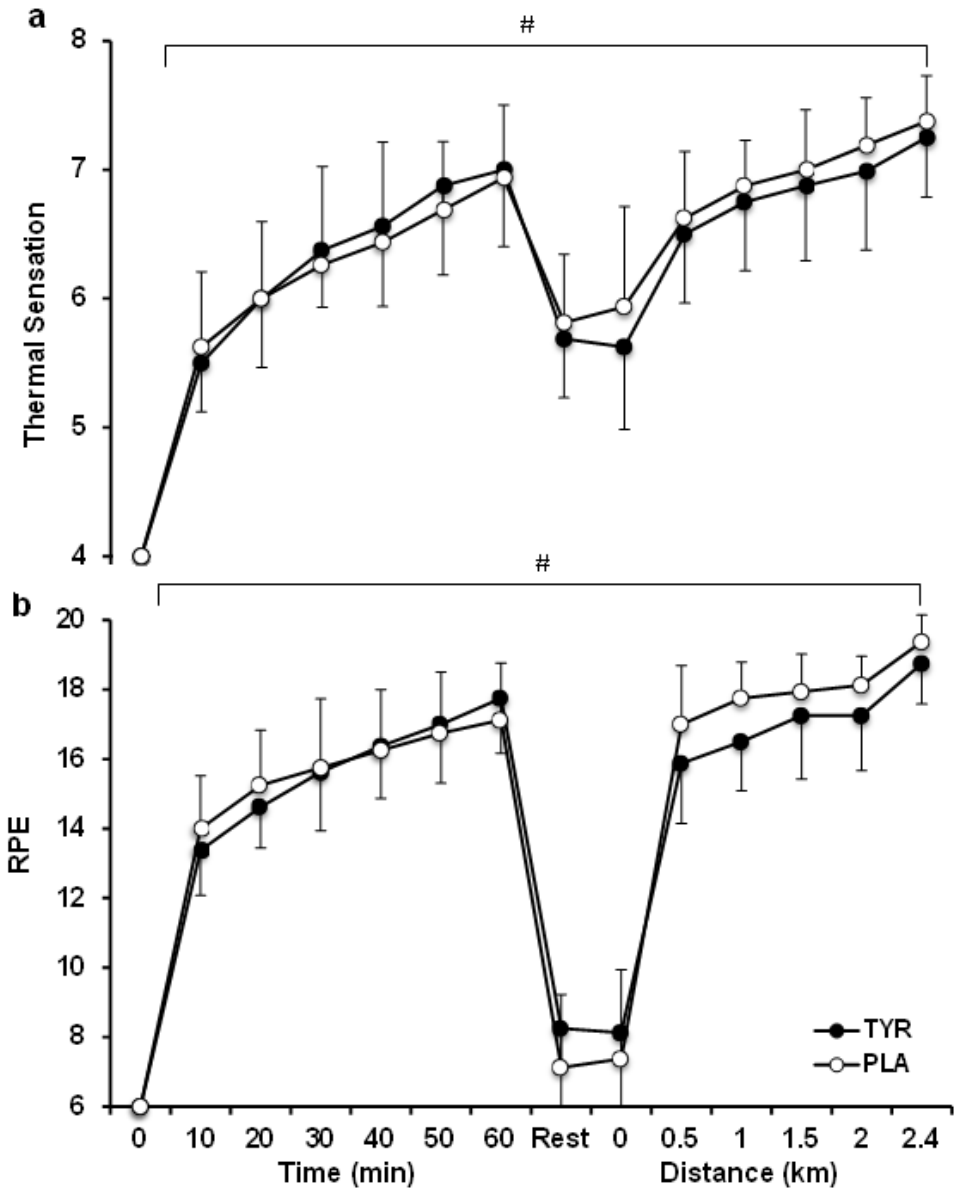

Figure 5.9. Group mean thermal sensation (TSS) (a) and rating of perceived exertion (RPE) (b) in TYR and PLA, during the 60 min walk, rest period and 2.4 km time-trial. Values are mean ± SD. #Significant differences over time (p < 0.05).

5.4.6 Effort scales

A significant effect of time for mean RTIPE was observed ($F_{7,105}$ = 38.41, p < 0.001), as on average RTIPE decreased by 77% and 75% from pre- to post-exercise in TYR and PLA, respectively (p < 0.001, 95% CI = 4.9-7.8) (Figure 5.10a). However, there was no significant main effect for condition ($F_{1,105}$ = 0.69, p = 0.41) between TYR (5 ± 1) and PLA (5 ± 1) and no significant condition x time interaction effect ($F_{7,105}$ = 0.90, p = 0.51). Equally, a significant effect of time was observed for mean RTIME ($F_{7,105}$ = 32.24, p < 0.001), with a 75% and 74% decrease from pre- to post-exercise in TYR and PLA, respectively (p < 0.001, 95% CI = 4.7-7.7) (Figure 5.10b). No significant main effect of condition was observed ($F_{1,105}$ = 1.56, p = 0.22) between TYR (5 ± 1) and PLA (5 ± 1) and no significant condition x time interaction effect ($F_{7,105}$ = 0.93, p = 0.65).

5.4.7 Hydration status

No significant difference was observed in pre-exercise urine osmolality ($F_{1,14}$ = 0.01, p = 0.95) between TYR (141.3 ± 100 mOsm kg^{-1}) and PLA (145 ± 114 mOsm kg^{-1}). No significant difference was observed in mean sweat loss ($F_{1,14}$ = 0.03, p = 0.87), which was calculated from pre- to post-exercise body mass measurements, between TYR (1.7 ± 0.3 L) and PLA (1.6 ± 0.5 L).

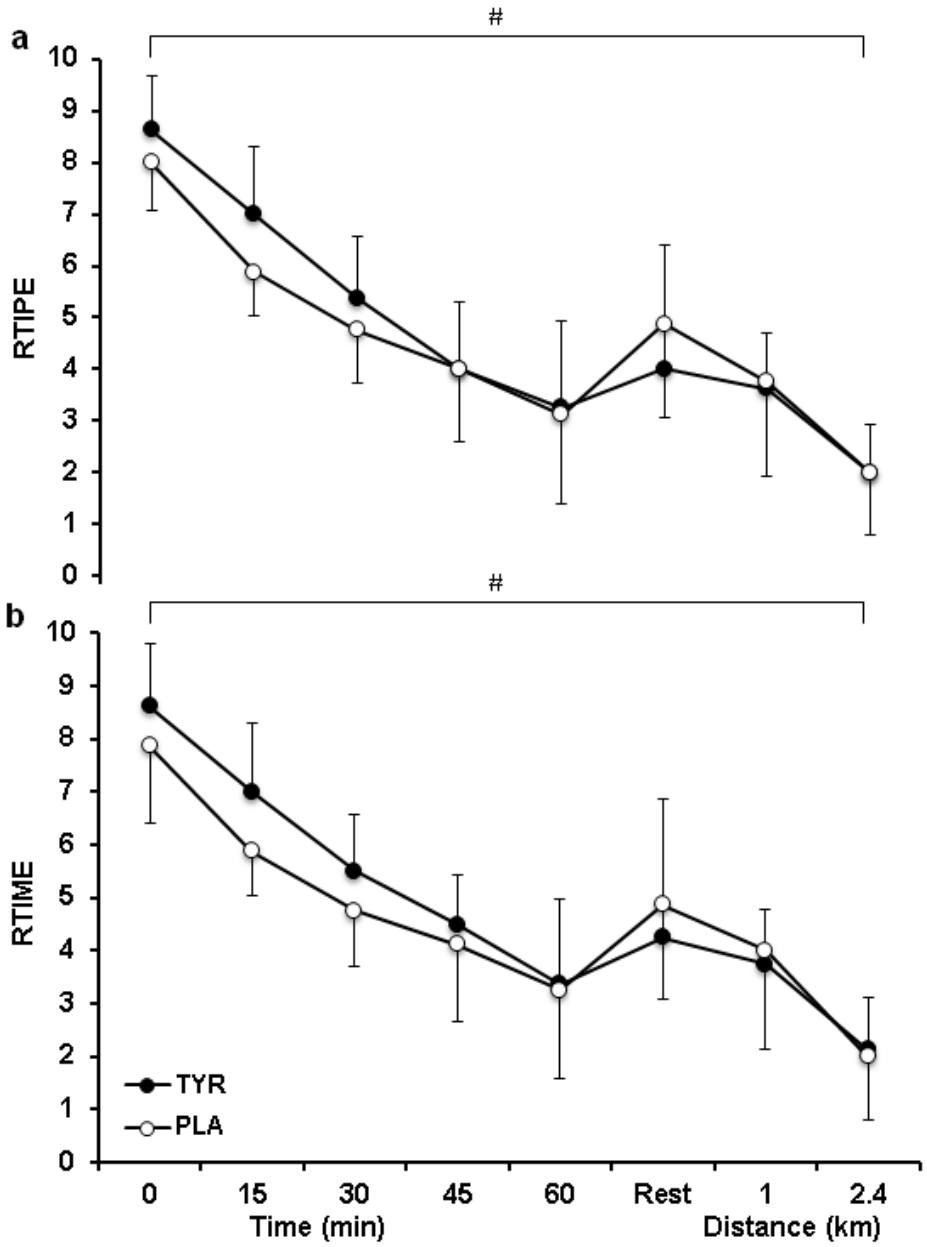

Figure 5.10. Group mean RTIPE (a) and RTIME (b) in TYR and PLA, during the 60 min walk, rest period and 2.4 km time-trial. Values are mean ± SD. #Significant differences over time ($p < 0.05$).

5.5 Discussion

The aim of this experimental chapter was to investigate the effects of TYR on steady state exercise, cognitive function and 2.4 km time-trial performance in a hot environment (40°C), utilising the identified dose from the findings of experimental chapter 1. The main finding was that 150 mg·kg body mass^{-1} TYR ingested 1 h pre-exercise did not significantly influence any aspect of cognitive function (vigilance, dual-task and simple reaction time), contrary to the primary hypothesis. In both conditions, a significant main effect of time ($p < 0.01$) was observed for FALSE scores (vigilance and dual-task), dual-task TRACKING scores and for simple reaction time, demonstrating declines in cognitive function both during and immediately after exercise-heat-stress from pre-exercise scores (Table 5.3). Furthermore, as expected TYR ingestion had no beneficial effect on physical performance, as time-trial time was similar in both conditions (Figure 5.6). Despite a large body of literature demonstrating that TYR is a useful ergogenic aid (Deijen *et al.*, 1999, Lieberman *et al.*, 2005, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Tumilty *et al.*, 2011, Kishore *et al.*, 2013, Coull *et al.*, 2015), the present findings are in accordance with two recent studies suggesting that 150 mg·kg body mass^{-1} TYR does not influence cognitive function and/or physical performance in hot environmental conditions (Watson *et al.*, 2012, Tumilty *et al.*, 2014).

Within humans the attainment of a state of 'central fatigue' via an intervention (exercise or otherwise) is problematic and debate still exists regarding the methods used to indicate whether such a state has occurred. Future work is evidently required to find and develop valid and reliable metrics to identify a state of 'central fatigue' in humans. The current protocol was employed on the basis that the combination of load-carriage (25 kg) exercise and extreme heat exposure (40°C) would create an ecologically valid and sufficiently demanding environment to alter central catecholamine neurotransmission. During acute stress, there is an observed increase in the activation of noradrenergic neurons in the frontal cortex, which release neurotransmitter as a response to stress (Deijen and Orlebeke, 1994). The continued release of neurotransmitter is fundamental in the ability to cope with stress, and thus as concentrations begin to deplete, aspects of cognitive function start to deteriorate (Kishore *et al.*, 2013). Hence, the use of TYR to increase catecholamine synthesis is

believed to be useful in this state and has proved to be beneficial in previous studies reporting improvements in aspects of cognitive function (vigilance, working memory, tracking) and mood in stressful conditions (Neri *et al.*, 1995, Deijen *et al.*, 1999, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Kishore *et al.*, 2013, Coull *et al.*, 2015). Conversely, in the present study this was not the case, as unexpectedly, TYR did not influence vigilance, dual-task or simple reaction time, despite the sound neurochemical basis for its use. This was despite observed impairments in cognitive function after the exercise-heat-stress (see Table 5.3), suggesting that the experimental protocol was sufficiently demanding, as anticipated. Moreover, in support of this, end HR (189.5 ± 4.2 v 197.9 ± 7.4 b min $^{-1}$), RPE (18.8 ± 1.2 v 19.4 ± 0.7) and TSS (7.3 ± 0.5 v 7.4 ± 0.4) values reached near maximum and post time-trial T_{re} was also high (38.8 ± 0.4 v 38.8 ± 0.5°C) in both TYR and PLA conditions respectively.

Although the present protocol was physically demanding, it could be argued that the combination of multiple stressors experienced in previous military based studies (Banderet and Lieberman, 1989, Deijen and Orlebeke, 1994, Deijen *et al.*, 1999, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007) may have induced more severe stress-related decrements in cognitive function and mood state. For example, in one study, military personnel endured a weeklong combat training course in which they were physically and emotionally stressed and also deprived of sleep and food (Deijen *et al.*, 1999). Such prolonged stress may have caused significant depletion in central catecholamine concentration and thus provision of exogenous TYR may be more likely to exert beneficial effects. It may be that exercise-heat-stress alone is not sufficient enough to manipulate central catecholamine activity to an extent whereby TYR is influential. However, as the military studies are mostly field based, it is difficult to compare these findings with the present laboratory controlled data.

The present study utilised the exact same cognitive test software (PsychE) as a recent TYR investigation by Coull *et al.* (2015), who observed improvements in vigilance when exposed to a demanding soccer-simulation in a warm environment (25°C) (see Appendix G for full study details). The exercise-heat-stress experienced by participants in the present study was similar, if not more stressful than that of Coull *et al.* (2015), when comparing similar data such as end HR (175 b min $^{-1}$ in TYR and

177 b min $^{-1}$ in PLA), T_{re} (38.7°C in TYR and 39.0°C in PLA), RPE (16.6 in TYR and 17.1 in PLA) and TSS (6.8 in TYR and 7.1 in PLA) (see above for data from the present study). Thus it is surprising that TYR did not have similar beneficial effects on cognitive function (improved vigilance). One possible explanation for this is that the cognitive tests employed within both studies (the present study and Coull *et al.* (2015)), are not necessarily 100% reliable according to the reliability statistics within the present thesis (Table 5.1). It appears from the available data that vigilance MISS and FALSE scores and dual-task FALSE scores are unreliable and therefore this must be taken into account when comparing findings between studies, as intra-individual and inter-individual differences in cognitive tests responses will influence the level of significance.

Another possible reason for the opposing findings between the two studies may be the differing levels of psychological stress experienced. Since psychological stress is also associated with neurotransmitter depletion, small differences in the cognitive demand on the participants in each study may influence the level to which TYR has an effect (Jongkees *et al.*, 2015). During the soccer simulation protocol utilised by Coull *et al.* (2015), participants had to constantly focus on specific audio and visual cues from the computer software, instructing them to change intensity, for a total of 90 min (see Appendix G methodology section for details). This added cognitive demand may have increased the psychological stress experienced by the participants. Whereas in the present investigation participants completed a 60 min walk with no added cognitive demand besides the 10 min cognitive test battery at the halfway point. Therefore, it could be speculated that the added psychological stress in the study by Coull *et al.* (2015) may have increased the potential for TYR to influence performance. The author acknowledges that blood measures of TYR, LNAA, DA and NA would have strengthened the study and allowed solid conclusions to be made regarding the opposing findings. However, this was not possible within the present investigation and is a potential avenue for future research in line with Kishore *et al.* (2013).

Moreover, differences in the methodologies between these two studies may partially explain the opposing findings. For, example, the present study administered a single dose of TYR, compared to the double dose utilised by Coull *et al.* (2015), however as observed in experimental chapter 1, a double dose may not necessarily result in

significantly higher concentrations of circulating TYR. Furthermore, it is also worth noting that the supplement administered in the present study was sourced from a medical nutrition supplier (Nutricia Ltd), whereas the TYR used by Coull *et al.* (2015) was obtained from an online sport nutrition company (Myprotein.co.uk). Due to the known uncertainty regarding the composition of widely available nutritional supplements in the field, this is important to consider (Maughan, 2005, Watson *et al.*, 2012). The exact composition of both supplements is not exactly known, however HPLC analysis of the amino acid concentration revealed that the medical nutrition supplement was >98% pure, whereas the supplement sourced from the sport nutrition company only contained ~90% TYR (Coull *et al.*, 2015). The difference between the two supplements may be a possible explanation for the observed findings, especially as two other studies who also used a verified form of TYR from a medical nutrition company (SHS International), failed to find improvements in cognitive function or exercise performance in the heat (30°C; 50% RH) (Watson *et al.*, 2012, Tumilty *et al.*, 2014). It is possible that the remaining 8% composition of the online sport nutrition supplement may contain an unknown substance(s) that could potentially influence performance (Maughan, 2005), however this is not clear from the analysis.

The present study also assessed perceptual responses of effort using a readiness to invest effort scale (RTIPE and RTIME) in line with previous studies (Duncan *et al.*, 2012, Coull *et al.*, 2015). Ratings of RTIPE and RTIME significantly decreased during exercise-heat-stress in both TYR and PLA trials (Figure 5.10), as anticipated. However, ingestion of TYR did not influence these ratings, conflicting the recent data (Appendix G) from (Coull *et al.*, 2015). As TYR has consistently been shown to improve aspects of mood and motivation (discussed previously), it was predicted that TYR would improve RTIME and/or RTIPE. It must be noted that the scales used within the current study are not necessarily a valid and reliable measure of mood, however they enable immediate responses, appropriate for assessment during exercise. Retrospectively, the use of the well-established profile of mood states (POMS) questionnaire could have been used alongside (but not a replacement of) these perceptual measures for a pre-to post-exercise mood state comparison, similar to previous studies (Banderet and Lieberman, 1989, Mahoney *et al.*, 2007, Piacentini and Meeusen, 2015).

As expected, TYR did not influence physical performance in the present study, with no difference in 2.4 km time-trial time between conditions. To date, only one study has observed a beneficial effect of TYR (Myprotein.co.uk) on physical performance, with an observed 15 ± 11% increase in exercise capacity in the heat (30°C; 50% RH), compared to placebo (Tumilty *et al.*, 2011). As the TYR used in this study (Tumilty *et al.*, 2011) was sourced from the same company as Coull *et al.* (2015) it may be assumed both supplements have a similar composition and thus these findings should be interpreted with care. Conversely, all other studies (including the present study) assessing the effects of TYR on exercise performance, have failed to demonstrate significant performance improvements in temperate (Strüder *et al.*, 1998, Chinevere *et al.*, 2002, Sutton *et al.*, 2005) and hot environmental conditions (Watson *et al.*, 2012, Tumilty *et al.*, 2014, Coull *et al.*, 2015). In temperate conditions, these findings are not surprising as under conditions that are not highly stressful, cerebral levels of TYR hydroxylase are saturated with substrate, thus supplementing with TYR should not, in theory, increase catecholamine synthesis (Lehnert *et al.*, 1984, Foley and Fleshner, 2008). However, during exercise-heat-stress there is a strong rationale for the use of TYR due to the observed depletion in neurotransmitter release during stress and the consequential reduction in motor control, arousal and motivation (Lehnert *et al.*, 1984, Lieberman *et al.*, 2005). Tumilty *et al.* (2014) propose a possible mechanism to elucidate the lack of beneficial effects, suggesting that ingestion of TYR may have increased central NA activity and thus counteracted any influence on performance. This theory comes after one study demonstrated a reduction in exercise performance after ingestion of a NA reuptake inhibitor (reboxetine) in a hot environment (Roelands *et al.*, 2008). As TYR is a general catecholamine precursor, this explanation is plausible, however further investigation into the central effects of TYR are required.

There are several limitations within the present investigation, which may be considered for future research in this area. One obvious issue is the recruitment of recreationally active male volunteers, rather than military personnel. The fact that the participants had no prior experience with load-carriage or military based protocols may have confounded results. However, as all participants had rigorous familiarisation with the backpack and load-carriage protocol (see section 5.3.3), the influence of this issue was likely therefore minimised. Furthermore, the protocol

speed (6.5 $km.h^{-1}$) and backpack mass (25 kg) was not individualised for each participant, thus the physiological stress may not have been equal which may have caused inter-individual differences in the dependant variables measured. The current protocol closely simulates training exercises and assessments used within the British Army and is similar to the Infantry Basic Combat Fitness Test (8 mile course at 15 min per mile (6.4 $km.h^{-1}$), carrying a 25 kg load) and Advanced Combat Fitness Test 1 (2.4 km time-trial with 20 kg load) (Knapik and Reynolds, 2012, Faghy and Brown, 2014a). Thus, to preserve the validity of the protocol in relation to military operations, it was not individualised and instead recruitment of a homogenous sample of participants (see section 5.3.1 for participant characteristics) was attempted, to minimise inter-individual variation.

Finally, the cognitive test software utilised in the present study was laptop based and only measured certain aspects of cognitive function. Two of the tests also lacked reliability in some instances and thus the present results must be interpreted with care. It may also be that the software was not sensitive enough to reveal small improvements and thus this investigation would have benefitted from a more advanced assessment of cognitive function. For example, a recent study assessed the effects of TYR on cognitive function during passive heat-stress and used event related potentials to provide a neurophysiological method of examining higher cerebral function (Kishore *et al.*, 2013). Other heat-based studies have utilised brain imaging techniques (fMRI and EEG) (Hocking *et al.*, 2001, Sun *et al.*, 2011, Liu *et al.*, 2013) and the attention network test (Sun *et al.*, 2011, Liu *et al.*, 2013) to broadly assess higher levels of cognitive function and brain activity simultaneously. Future studies examining cognitive function should utilise these objective tools as they provide a reliable, quantifiable and non-invasive method of measuring the brain activity responsible for certain cognitive processes (Kishore *et al.*, 2013). However, it must be noted that such advanced techniques may be difficult, if not impossible to implement during exercise.

5.6 Conclusion

In conclusion, the results of the present study demonstrate that load-carriage (25 kg) in a hot environment (40°C) significantly impairs aspects of cognitive function compared to pre-exercise measures and contrary to the experimental hypothesis

ingestion of 150 mg·kg body mass^{-1} TYR did not attenuate these decrements. Furthermore, TYR did not influence 2.4 km time-trial performance with similar completion times observed in both conditions, suggesting that under the conditions of the present study, TYR is not a useful ergogenic aid. Future research should aim to elucidate the central effects of TYR to enable a better understanding of its mechanistic properties.

CHAPTER 6: General Discussion and Conclusions

6.1 General Discussion

The aim of this thesis was to investigate the efficacy of TYR as an ergogenic aid by first assessing the pharmacokinetics of TYR ingestion and secondly examining the effect of the identified dose on physical performance and cognitive function in a hot environment (40°C), utilising a military based protocol (Faghy and Brown, 2014a). Experimental chapter 1 identified that a single dose of 150 mg·kg body mass^{-1} was sufficient to elevate serum TYR concentrations without the need for ingestion of a second dose, as seen previously (Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Coull *et al.*, 2015). Furthermore, administration of this dose did not induce any side effects and thus was considered safe to ingest prior to a stressful exposure.

Despite these findings, experimental chapter 2 demonstrated that ingestion of 150 mg·kg body mass^{-1} TYR did not significantly influence any aspect of performance. This is surprising since heat-related declines in cognitive function were apparent, suggesting that the load-carriage protocol was physically demanding, however contrary to the primary hypothesis and findings from previous research (Banderet and Lieberman, 1989, Deijen *et al.*, 1999, Mahoney *et al.*, 2007, O'Brien *et al.*, 2007, Kishore *et al.*, 2013, Coull *et al.*, 2015), TYR did not alleviate these decrements. Similarly, TYR did not influence physical performance as no differences were observed in response to steady state exercise (60 min walk) or 2.4 km time-trial performance time in the heat. This finding was to be expected as the majority of previous studies have also failed to observe any beneficial effect of TYR on aspects of physical performance in both temperate and hot environmental conditions.

A number of possible reasons may explain the findings of the present investigation, however one particularly interesting issue has been highlighted within this thesis. The source of the TYR supplement administered in the present and previous studies may be of great importance when comparing findings. Of the studies that have reported the source of their TYR supplement, two studies have observed significant improvements in cognitive function (Coull *et al.*, 2015) and exercise capacity (Tumilty *et al.*, 2011) in the heat after ingestion of TYR from an online sport nutrition company (Myprotein). Whereas the studies that have utilised a TYR supplement sourced from

verified medical nutrition companies (including the present study) have failed to find any benefit of ingesting TYR, despite similar elevations in blood TYR concentrations (Watson *et al.*, 2012, Tumilty *et al.*, 2014). As previously discussed, this is important to consider due to the known uncertainty with widely available sport nutrition products (Maughan, 2005). However, further research into the composition of both supplements is required before assumptions can be made.

6.2 Future Research Recommendations

Several limitations have been highlighted in relation to the investigations of experimental chapter 1 and 2 (see relevant chapter for experimental limitations), which should be considered for future research. In light of these limitations, it is recommended that future studies utilise strictly medical grade TYR supplements that have been sourced from verified medical nutrition suppliers to minimise the risk of contamination and to enable study findings to be easily compared. Furthermore, due to the known confounding factors that can influence cognitive function, simply employing basic cognitive test batteries is no longer sufficient enough to draw confident conclusions. Therefore, to elucidate the mechanistic action of TYR and heat-stress in general, the use of advanced brain imaging techniques in tandem with intuitive cognitive testing (e.g. attention network test) are encouraged to provide reliable, object measures of high-level cerebral activity.

6.3 Overall Conclusion

In summary, the findings presented within the present thesis provide useful pharmacokinetics data of the most commonly administered doses of TYR within the literature. However, despite observing marked elevations in serum TYR concentrations, the present findings demonstrate that 150 $mg \cdot kg$ body $mass^{-1}$ TYR had no beneficial effect on any aspect of cognitive function or physical performance. Therefore, this thesis provides further evidence to suggest that TYR does not influence physical performance as currently only one study has observed a beneficial effect to date. Conversely, as the majority of previous literature has demonstrated improvements in cognitive function after TYR ingestion, further research is required in this area before TYR is discounted as an ergogenic aid.

CHAPTER 7: References

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CHAPTER 8: Appendices

Appendix A

Information to Participants

Study Title:
Tyrosine Ingestion and its Effects on Cognitive Function and Load-Carriage Performance in the Heat

Lead Researcher: Nicole Coull BSc (Hons)
Supervisor: Dr. Lee Taylor, Ph.D
2nd Supervisor: Dr. Bryna Chrismas, Ph.D

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?
The purpose of the study is to investigate the effects of an acute dose of tyrosine (a non-essential amino-acid, naturally produced by the body) on serum levels of tyrosine and other large neutral amino acids in the blood. This study is being undertaken as part of the requirements of MSc by Research (MRes) degree at the University of Bedfordshire.

What type of participant is needed?
Recreationally active males aged between 18 and 30 years old.

What will participants be asked to do?
The participant will be required to attend the University of Bedfordshire laboratories on 5 separate occasions.

Study 1 (1 day): Visit 1 will involve baselines measures including height, weight and baseline blood samples taken. The participant is then required to consume the first dose of tyrosine/control depending on their allocation to either group 1 (CON; no tyrosine), group 2 (LOW; 2 doses of 75 mg/kg) or group 2 (HIGH; 2 doses of 150 mg/kg) and remain in the lab for further blood samples. Four hours post ingestion of the first dose of tyrosine, participants will consume the second dose. Participants will then rest for the remainder of the session, having blood samples taken every hour for 4 hours after ingestion of the second dose. During the time in the laboratory, participants will consume a set breakfast and lunch (cornflakes and semi-skimmed milk) provided as part of the study to control the intake of tyrosine within their food. On the day prior to the trial they will consume their normal diet and record this in a food diary. Participants will then fast from 9pm until the morning of the trial.

Study 2: Visit 2-5 will involve 2 familiarisation sessions in which participants will complete shortened bouts of the 6.5 km/h march and demo versions of the cognitive tests at rest and while walking on the treadmill in temperate conditions (20 °C) to accustom them to the protocol and to minimize a learning effect. A time trial (2.4k) will also be performed during familiarisation to accustom participants to self-paced treadmill exercise. A weighted backpack (25kg) will be worn during familiarisation and the main trials to simulate occupation settings.

As with study 1, participants will complete food diaries on the day before each experimental condition to then replicate on the subsequent visit. On the day of testing, participants will ingest the dose of TYR or PLA (150 mg/kg TYR or sugar free lemon squash for placebo) and rest in the laboratory for 1 hour prior to beginning the exercise protocol. Baseline measures will be taken (height, mass, urine and blood samples). Participants will be required to insert a pre-prepared rectal thermistor. Skin thermistors will then be applied to the participants at 4 sites as well as a heart rate monitor. After the necessary safety measures have been applied and the participant has rested for 5 min in a thermal neutral room (~18 ^{o}C),

the participant will then enter the environmental chamber (40 ^{o}C; 30% RH). Participants will then complete the load carriage protocol (60 min) followed by a 15 min rest period and then a 2.4 km time trial on the treadmill (40 ^{o}C; 30% RH) wearing a 25kg weighted backpack. Rectal temperature, skin temperature, heart rate, ratings of perceived exertion (Borg, 1982) and thermal sensation will be recorded every 5 min throughout the protocol and rest period to ensure the participants safety is not compromised (see potential risks for a detailed explanation). Cognitive function will also be measured before, during and after exercise on a computer-based software. Blood samples will be taken pre-exercise, during the 15 min rest period and after the time-trial via venepuncture.

What are the possible risks of taking part in the study?
The ingestion of tyrosine can cause side effects, such as headaches, heartburn, joint pain, seriously upset stomach or an allergic reaction; however these are very uncommon and if any of these symptoms occur, testing will be immediately stopped and the participant will receive medical help. The doses of tyrosine used in this study have previously been used in the University of Bedfordshire laboratories and have caused no harm to any participant and we consider the doses to be safe to ingest. **If you have, or if any of your family members have a history of hyperthyroidism (overactive thyroid) or a condition called Grave's disease then you should inform the researcher, as it is not safe for you to consume tyrosine supplements. Also if you are taking medication for thyroid hormone or taking levodopa then tyrosine is not recommended.**

There are also risks associated with exercising in the heat such as heat stroke and exertional heat illness, however these are very uncommon and will be minimized when carrying out the correct safety measures. Furthermore, to monitor core temperature a rectal thermometer must be inserted which may be uncomfortable or cause anaphalactoid shock, this is also very rare and should this happen, first aid will be provided immediately. Do not be alarmed after reading the possible risks of this study as these are very rare and the researcher is fully aware of all the possible risks (and fully first aid trained) and the participant will be in a safe environment when testing in the laboratory.

What if you decide you want to withdraw from the project?
If at any stage you wish to leave the project, then you can. There is no problem should you wish to stop taking part and it is entirely up to you. There will be no disadvantage to yourself should you wish to withdraw.

What will happen to the data and information collected?
Everyone that takes part in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?
Questions are always welcome and you should feel free to ask myself, Nicole Coull or Dr. Lee Taylor (supervisor) any questions at anytime. See details below for specific contact details.

Should you want to participate in this study then please complete the attached consent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

Many Thanks,

Nicole Coull BSc (Hons)
Tel: 07429096867
Email: Nicole.coull@study.beds.ac.uk

Dr. Lee Taylor
Email: Lee.taylor@beds.ac.uk

Appendix B

CONSENT FORM

TO BE COMPLETED BY PARTICIPANT

Name of participant:…… Date:……

I have read the Information Sheet concerning this project and understand what is required of me as a participant. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

- My participation in the project is entirely voluntary and I am free to withdraw from the project at any time without disadvantage or prejudice.
- I will be required to attend **5 sessions** to complete the project.

As part of the study I will have to:

- Ingest a safe acute dose of tyrosine (150 or 300 mg/kg).
- Have several blood samples taken to assess serum tyrosine levels via venepuncture and cannulation.
- Record 24-hour food diaries and replicate these when required.
- Consume a fixed diet of cornflakes and semi-skimmed milk.
- Have my body fat assesses via the Bod Pod.
- Exercise in heated conditions, complete cognitive tests and have physiological measures taken throughout the trials.
- Wear a 25 kg weighted backpack during the exercise protocol.
- Insert a rectal thermometer to monitor core temperature for the purpose of the study and for your own safety.

I am aware of any risks that may be involved with the project.

All information and data collected will be held securely at the University indefinitely. The results of the study may be published by anonymity will be persevered.

Signed:…….

Appendix C

BLOOD ANALYSIS – Participant Screening Form

Please read the following:

a. Are you suffering from any known active, serious infection?
b. Have you had jaundice within the previous year?
c. Have you ever had any form of hepatitis?
d. Have you any reason to think you may be HIV positive?
e. Have you ever been involved in intravenous drug use?
f. Are you a haemophiliac?
g. Is there any other reason you are aware of why taking blood might be hazardous to your health?
h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer **Yes** to any of questions a-g? Please tick your response in the box below:

Yes ☐ No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the researcher guidance as to my suitability for the test.

Name ……

Signed ……

Date ……

If there is any change in the circumstances outlined above, it is your responsibility to tell the person administering the test immediately.

This Blood Sampling Form will be held in a locked filing cabinet in the Department of Sport and Exercise Science laboratories at the University for a period of one-three years. After that time all documentation will be destroyed by shredding.

Appendix D

Risk Assessment Questionnaire

Name:…… Date:……

Please read the following carefully and answer as accurately as possible. The questions are designed solely to determine whether the proposed protocol is safe for you. Your answers will be treated as strictly confidential. If you have any doubts or difficulties with any of the questions please contact the researcher.

1. Are you allergic to dairy, corn or soybeans (ingredients in cornflakes)?
   If so please state below.

   YES …….. NO

2. Do you have allergies to tyrosine?

   YES NO

3. Do you have any medical conditions related to thyroid problems? (E.g. overactive thyroid or Grave's disease).

   YES NO

4. Do you know of any family members who you have any medical conditions related to thyroid problems? (E.g. overactive thyroid or Grave's disease).

   YES NO

5. Do you have Phenylketonuria (PKU)?

   YES NO

6. Do you currently take any medication or supplements? If yes, please specify.

   YES NO

7. Is there any issue not raised that may mean that it is not safe for you to take tyrosine? Please state below.

   YES NO

I have completed the questionnaire to the best of my knowledge and any questions that I have raised have been answered to my full satisfaction.

Signed:…….

Appendix E

Participant reminders prior to your trial – Please read carefully

IT IS IMPORTANT YOU FOLLOW THESE STEPS OR YOUR RESULTS WILL BE INVALID

- Please do not exercise in the 24 hours leading up to your trial.

- Please do not consume alcohol or caffeine for the 24 hours leading up to your trial.

- **On the day before** your lab visit, record your food and drink consumption (24 hours) on the food diary provided – ensure you eat meals that you would like to replicate.

- **VISIT 1 ONLY:** Please do not eat or drink anything other than water after 9pm on the day before your lab visit.

- Drink at least 500 mL of water on the morning before all visits to ensure you are hydrated.

- Please wear the same clothing/trainers on all laboratory visits.

Appendix F

Pre-test questionnaire

Honestly answer these few questions before performing the experimental tests.

Name: …….

- Have you consumed alcohol or caffeine in the last 24 hours?

  YES NO

- Have you taken part in exhaustive exercise in the last 24 hours?

  YES NO

- Have you completed and brought with you a 24 hour food diary?

  YES NO

- Have you ingested any supplements in the last 4 weeks (including protein, BCAA, caffeine supplements etc.)?

  YES NO

- Approximately how many hours of sleep did you get last night

Appendix G - Previous TYR work conducted by our research group published in European Journal of Applied Physiology (Coull *et al.*, 2015).

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ORIGINAL ARTICLE

Effect of tyrosine ingestion on cognitive and physical performance utilising an intermittent soccer performance test (iSPT) in a warm environment

Nicole A. Coull · Samuel L. Watkins · Jeffrey W. F. Aldous · Lee K. Warren · Bryna C. R. Chrismas · Benjamin Dascombe · Alexis R. Mauger · Grant Abt · Lee Taylor

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Abstract
Purpose The aim of this study was to investigate the effect of tyrosine (TYR) ingestion on cognitive and physical performance during soccer-specific exercise in a warm environment.
Methods Eight male soccer players completed an individualised 90 min soccer-simulation intermittent soccer performance test (iSPT), on a non-motorised treadmill, on two occasions, within an environmental chamber (25 °C, 40 % RH). Participants ingested tyrosine (TYR; 250 mL sugar free drink plus 150 mg kg body mass^{-1} TYR) at both 5 h and 1 h pre-exercise or a placebo control (PLA; 250 mL sugar free drink only) in a double-blind, randomised, crossover design. Cognitive performance (vigilance and dual-task) and perceived readiness to invest physical effort (RTIPE) and mental effort (RTIME) were assessed: pre-exercise, half-time, end of half-time and immediately post-exercise. Physical performance was assessed using the total distance covered in both halves of iSPT.
Results Positive vigilance responses (HIT) were significantly higher (12.6 ± 1.7 vs 11.5 ± 2.4, $p = 0.015$) with negative responses (MISS) significantly lower (2.4 ± 1.8 vs 3.5 ± 2.4, $p = 0.013$) in TYR compared to PLA. RTIME scores were significantly higher in the TYR trial when compared to PLA (6.7 ± 1.2 vs 5.9 ± 1.2, $p = 0.039$). TYR had no significant ($p > 0.05$) influence on any other cognitive or physical performance measure.
Conclusion The results show that TYR ingestion is associated with improved vigilance and RTIME when exposed to individualised soccer-specific exercise (iSPT) in a warm environment. This suggests that increasing the availability of TYR may improve cognitive function during exposure to exercise-heat stress.

Keywords Central fatigue · Tyrosine · Cognitive function · Intermittent exercise · Heat

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N. A. Coull · S. L. Watkins · J. W. F. Aldous · L. K. Warren · B. C. R. Chrismas · L. Taylor (✉)
Department of Sport Science and Physical Activity, Institute for Sport and Physical Activity Research (ISPAR), University of Bedfordshire, Bedford, UK
e-mail: Lee.taylor@beds.ac.uk

B. Dascombe
Applied Sports Science and Exercise Testing (ASSET) Laboratory, Faculty of Science and IT, University of Newcastle, Callaghan, Australia

A. R. Mauger
Endurance Research Group, School of Sport and Exercise Sciences, University of Kent, Chatham Maritime, Kent, UK

G. Abt
Department of Sport, Health and Exercise Science, University of Hull, Hull, UK

Abbreviations

5-HT	Serotonin
CNS	Central nervous system
DA	Dopamine
HR	Heart rate
iSPT	Intermittent soccer performance test
LNAA	Large neutral amino acids
NA	Noradrenaline
NMT	Non-motorised treadmill
PLA	Placebo
RH	Relative humidity
RPE	Rating of perceived exertion
RTIME/RTIPE	Readiness to invest mental/physical effort

TSS	Thermal sensation
TYR	Tyrosine

Introduction

Soccer is the most widely played team sport in the world and is characterised as high-intensity, intermittent exercise, performed over a 90 min period (Stølen et al. 2005). Successful performance in soccer is dependent upon the simultaneous execution of technical, physical and mental skills (Meeusen et al. 2006a). However, the demanding, intermittent nature of the sport places players under high physiological strain and as a consequence, the ability to perform high-intensity exercise and maintain cognitive function declines towards the end of a match, due to the development of fatigue (defined as the inability to maintain work at a given intensity) (Bangsbo et al. 2006). The outcome of the game is highly dependent upon the ability of the players to cope with this fatigue and maintain physical and cognitive performance (Özgünen et al. 2010). This is reinforced by statistics from the European Soccer Championships (2004) demonstrating that a significantly higher percentage of goals were scored in the later stages of the second half (57.4 %) compared to the first half (42.6 %) (Yiannakos and Armatas 2006), attributed to lapses in concentration and mental fatigue in the opposing team (Reilly 1997).

Competitive soccer is often played in hot environments by recreational and elite players alike (Özgünen et al. 2010), imposing an additional stress on the body (exercise-heat stress). This added stress can accelerate the onset of fatigue (Mohr et al. 2012), progressively impairing exercise performance (Gonzalez-Alonso et al. 1999; Nybo et al. 2014) and cognitive function (Maughan et al. 2007; Simmons et al. 2008; Gaoua et al. 2011). Previous research has focused on peripheral mechanisms of fatigue, suggesting endogenous substrate depletion is the primary cause (Galloway and Maughan 1997; Bangsbo et al. 2006) however, it is now clear that there is also a significant involvement of the central nervous system (CNS) and psychological factors (Nybo et al. 2014). This shows that fatigue is a complex phenomenon, occurring at all levels of the brain–muscle pathway (Roelands and Meeusen 2010).

There are several theories of central fatigue (Cheung and Sleivert 2004) however, the original central fatigue hypothesis is based on the concept that during prolonged exercise, the activity and synthesis of the central monoamines are altered, specifically serotonin (5-HT), dopamine (DA) and noradrenaline (NA) (Newsholme et al. 1987; Meeusen et al. 2006b). An increased ratio of brain DA:5-HT is suggested to augment performance during prolonged exercise while low ratios induce lethargy and losses in motivation (Davis and Bailey 1997). Therefore, DA and NA are considered important neurotransmitters involved in both physical and cognitive performance due to their direct association with changes in arousal, motivation and motor control (McMorris et al. 2006; Watson 2008). Conversely, opposing evidence suggests that an increase in central NA decreases performance in the heat, as demonstrated by Roelands et al. (2008). During exercise, there is an elevation in concentrations of central catecholamine neurotransmitters in several cerebral regions, observed in the striatum and hypothalamus of rodents (Meeusen et al. 1997; Foley and Fleshner 2008). However, at the point of exhaustion, brain–tissue DA content (in rodents) is markedly decreased (Bailey et al. 1993), suggesting that the DA availability may be a possible mechanism for exercise-induced fatigue (Watson et al. 2012). This knowledge proffers the opportunity to manipulate the CNS with nutritional and pharmacological intervention strategies to attenuate the onset of fatigue during soccer match-play.

Many nutritional manipulation strategies are utilised in soccer (Nedelec et al. 2013) as small dietary mediated improvements in performance could significantly alter game outcome, by providing players with an advantage over their opponents. The precursor for catecholamine synthesis is TYR, a non-essential amino acid found in protein-rich dietary sources and synthesised in the liver from phenylalanine (Wurtman et al. 1980). Supplementation of TYR increases its ratio to other large neutral amino acids (LNAA) for competitive transport across the blood–brain-barrier, thus resulting in a greater cerebral uptake and an increase in DA and NA synthesis (Fernstrom and Faller 1978; Gibson and Wurtman 1978). Previous research involving TYR (100–300 mg kg body mass^{-1}) is primarily military based, finding improvements in certain aspects of cognitive function after exposure to stressful environments such as cold (Banderet and Lieberman 1989; Mahoney et al. 2007; O'Brien et al. 2007) and hypoxia (Banderet and Lieberman 1989), and paradigms involving both extended wakefulness (Neri et al. 1995) and the physical/emotional stress nexus (Deijen et al. 1999). Specific to hot environments, Tumilty et al. (2011) demonstrated a 15 ± 11 % increase in exercise capacity during constant-load, continuous cycling in the heat (30 °C; 50 % RH) after TYR ingestion (150 mg kg body mass^{-1}). However, to date, this is the first and only study to observe a beneficial effect of TYR on physical performance (with or without heat stress) in humans. More recently, similar studies have failed to replicate this finding during exercise to exhaustion (Watson et al. 2012) and a self-paced time trial (Tumilty et al. 2014) in the heat, despite the same dosage strategy and comparable increase in circulating TYR.

It appears that supplementing with TYR may alleviate stress-related decrements in cognitive function and possibly offset the perception of fatigue during exposure to

demanding environments. It is yet to be elucidated whether TYR has a positive effect on physical and cognitive performance aspects during soccer-specific exercise. Therefore, the aim of the present study was to investigate the effect of acute TYR ingestion on both cognitive and physical performance utilising an individualised, valid and reliable non-motorised treadmill (NMT) based soccer simulation (iSPT) (Aldous et al. 2014), in a warm environment (25 °C). It was hypothesised that a dose of 300 mg kg body mass^{-1} TYR would improve cognitive performance and increase the distance covered during iSPT, when compared to placebo.

Methods

Subjects

Eight healthy, University level soccer players (mean age 21 ± 1 years, height 180.3 ± 6.2 cm, body mass 74.9 ± 8.7 kg, body fat percentage 11 ± 5 % and physical activity 6.3 ± 1.2 h week^{-1}) volunteered to participate in this study. Prior to participation, subjects received detailed information about the study and subsequently provided their written informed consent. Subjects were not acclimated to exercising in the heat and had never consumed a supplementary dose of TYR before this study. Ethical approval was gained from the University of Bedfordshire Research Ethics Committee.

Familiarisation

Subjects were required to attend three familiarisation sessions prior to the experimental trials, involving shortened bouts of the soccer-simulation protocol (iSPT) (Aldous et al. 2014) on a NMT (Woodway, Force 3.0, Cranlea, Birmingham) in temperate conditions (18 °C). The activity pattern of the iSPT protocol is based on previous soccer match-play data and involves several movement categories (stand, walk, jog, run, fast run, variable run and sprint) (Aldous et al. 2014). Rigorous familiarisation [described in full in Aldous et al. (2014)] to iSPT ensured movement categories were individualised to each participant's sprint speed determined from a peak speed assessment on the NMT. Additionally, subjects were familiarised to the visual and audio cues presented to them by a computer program (Innervation, Pacer Performance System Software), which displayed their actual speed (red line) and a target speed (green line) that they were instructed to follow as closely as possible. Within these sessions, subjects also performed two demonstration versions of the vigilance and dual-task cognitive Psyche software tests (Hope et al. 1998). The familiarisation sessions were employed to ensure that subjects were accustomed to the protocol and were deemed appropriate to minimize any learning effects of the cognitive assessments (Hope et al. 1998) and iSPT (Aldous et al. 2014). Once familiarised with the protocol, subjects returned to the laboratory in a fasted state to have their body fat percentage assessed utilising bioelectrical impedance (Body Composition, Tanita, BC41MA Segmental Body, Cranlea).

Experimental procedure

During the experimental trials, each subject attended the laboratory on two separate occasions with at least 7 days between visits. Subjects refrained from alcohol, caffeine and unaccustomed exercise 24 h prior to the testing and completed food diaries to ensure replication of food intake prior to each performance of iSPT, in line with previous research in this area (Chinevere et al. 2002; Tumilty et al. 2011; Watson et al. 2012). Experimental controls were monitored via a questionnaire, with adherence confirmed at 100 % in all instances.

Upon arrival at the laboratory between 0700 and 0900, subjects orally ingested the first dose of either placebo [PLA (250 mL sugar free lemon squash) (Tesco, Bedford, UK)] or tyrosine [TYR (same as PLA plus 150 mg kg body mass^{-1} TYR powder) (Myprotein.co.uk)]. After a 4 h rest period subjects ingested an identical second dose (300 mg kg body mass^{-1} TYR in total) between 1100 and 1300 and then rested for 1 h prior to the start of the protocol. The drinks were prepared and coded by a separate laboratory technician to ensure that they were administered in a double-blind, randomised fashion. The drinks were provided in opaque sports bottles and were indistinguishable in taste and texture to the subjects. Prior pilot work confirmed that the dose of TYR administered in this study did not induce any side effects and this administration strategy has previously been shown to alleviate cold-induced decrements in psychomotor performance (O'Brien et al. 2007) and working memory (Mahoney et al. 2007). The TYR supplement used in the present study was analysed via high-performance liquid chromatography (HPLC) to assess its purity [using the method described by Watson et al. (2012)] and was found to contain a high concentration of TYR (>90 %).

Prior to exercise, nude body mass (Scales, Tanita, BWBO800, Allied Weighing) and height (Stadiometre, Harpenden, HAR-98-602, Holtain) were recorded and a urine sample was provided by the subject to assess hydration status using a urine refractometer (Atago Vitech scientific, Pocket PAL-OSMO, HaB Direct). Subjects were instructed to drink 500 mL of water 2 h prior to exercise in line with Sawka et al. (2007) and were deemed euhydrated if urine osmolality was <600 mOsm kg^{-1} H_2O (Hillman et al. 2011, 2013). This experimental control was not

Table 1 Descriptions of each cognitive test response (HIT, MISS, FALSE and TRACKING) for vigilance and dual-task assessments

Response	Vigilance	Dual-task
HIT	Correctly identifying a duplicate number	N/A
MISS	Failing to identify a duplicate number	Failing to identify a present icon
FALSE	Incorrectly identifying a duplicate number	Incorrectly identifying an icon is present when it is not
TRACKING	N/A	Ability to track a moving target (%)

breached prior to any experimental procedure commencing. A heart rate monitor (Polar, FS1, Cranlea) was attached and a rectal thermometer (Henleys, 400H and 4491H) inserted 10 cm past the anal sphincter. Skin temperature probes (Grant, EUS-U-VS5-0, Wessex Power) were attached to four skin sites: upper arm, chest, thigh and lower leg, using adhesive tape (Ramanathan 1964). Specific data loggers were used to record rectal (Libra Medical, ET402, Cranlea) and skin (Grant, Squirrel Series, model 451, Wessex Power) temperature. Subjects then entered the custom built Environmental Chamber set at 25 °C and 40 % RH, where they completed the cognitive assessments (vigilance and dual task) at rest.

The vigilance tests (2 min in duration) involved a number display where three–digit numbers flashed up on a laptop screen at a rate of 100 per min with an 8 % duplication rate. Subjects pressed the spacebar when a duplicated number appeared twice in a row and were scored on the amount of HIT (correct response), MISS (missed cue) and FALSE (false response) scores they achieved. The dual-task tests (3 min duration) required subjects to track a moving target with the mouse cursor and simultaneously respond to random stimuli with the spacebar. The percentages of time on the target (TRACKING) and stimuli responses (MISS and FALSE) were recorded. On completion of both tests, a report was provided, detailing the subject's scores for each test. All cognitive tasks were computer based and delivered in line with previous work in the field (Hope et al. 1998). See Table 1 for further details and description of the vigilance and dual-task assessments.

Measurements

Subsequent to the initial cognitive assessments, subjects rested for 5 min and pre-exercise measures were taken, including heart rate (HR), thermal sensation (TSS) using the 0–8 scale (Young et al. 1987), rating of perceived exertion (RPE) using the 6–20 Borg scale (Borg 1982), skin temperature (T_{sk}) of all four skin sites, rectal temperature (T_{re}) and readiness to invest physical effort (RTIPE) (Duncan et al. 2012) and mental effort (RTIME) (Duncan et al. 2012) using a 0–10 scale (see Duncan et al. (2012) for specific details of scale).

During iSPT [2 × 45 min halves, interspersed with the half-time period (15 min)], HR, RPE, TSS, T_{re} and T_{sk} were recorded every 5 min and the temperature and humidity of the chamber were recorded continuously. Weighted mean T_{sk} was calculated using the temperatures recorded for all four skin sites, using the following equation ('t' represents temperature):

$$0.3(t_{\text{chest}} + t_{\text{arm}}) + 0.2(t_{\text{thigh}} + t_{\text{leg}})$$

(Ramanathan 1964).

Cognitive function (vigilance and dual-task) and RTIPE and RTIME were assessed at four time points [pre exercise (0 min), onset of half-time (45 min), end of half time (EOHT) and immediately post exercise (90 min)], while subjects were seated in the chamber during the rest periods. Physical performance was assessed using the total distance covered during the first and second half of iSPT in both conditions. All subjects consumed a standardised amount of plain water (250 mL) during the 15 min half-time period and sweat losses were calculated from the difference in pre- and post-exercise body mass, after adjusting for any fluid consumed or urine excreted.

Statistical analyses

Statistical analyses were completed using IBM SPSS statistics 19.0 (IMB, Corporation, New York). Statistical assumptions were assessed using conventional graphical methods (Grafen et al. 2002) and deemed plausible for each variable. A two-way ANOVA (condition × time) with repeated measures was used to analyse mean differences in cognitive data, distance covered and all physiological, perceptual and thermoregulatory data between conditions (TYR and PLA). Where significance was obtained, Bonferroni post hoc tests were carried out. Assumptions of homogeneity of variance were assessed using Mauchly's test of Sphericity. Dual-task tracking and false scores violated sphericity ($p < 0.05$); therefore a Greenhouse-Geisser correction was applied to the degrees of freedom of the F ratio. Paired samples t-tests were performed to analyse the differences in sweat loss and pre-exercise urine osmolality between conditions. Two-tailed statistical significance was accepted at the $p < 0.05$ level. All data are presented as mean ± standard deviation (SD).

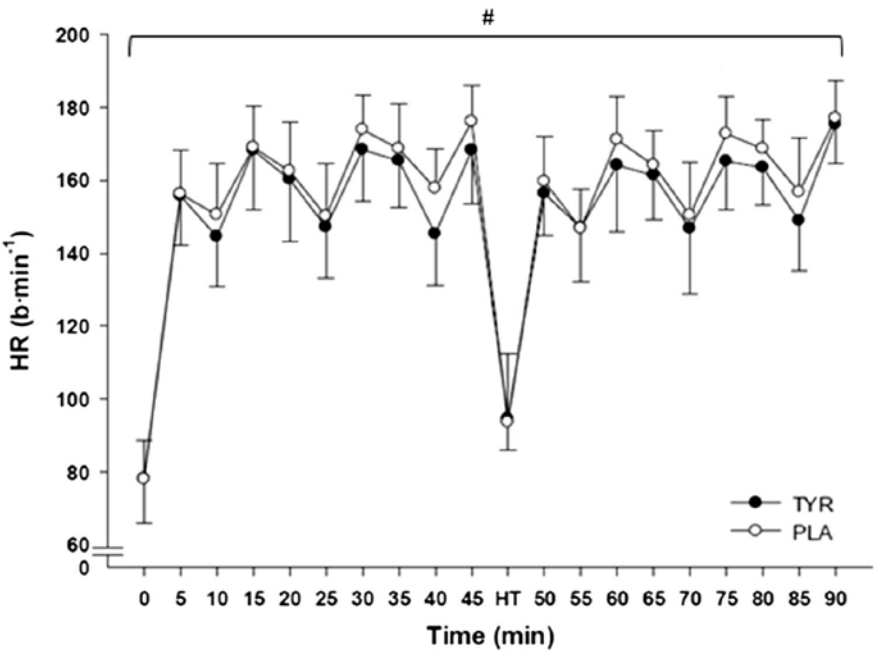

Fig. 1 Group mean heart rate (HR) (b min^{-1}) responses across both TYR and PLA conditions. Values are mean ± SD. Participants experienced similar increases in HR during both conditions that were not significantly different. #Significant differences over the 90 min protocol ($p < 0.05$)

Results

Hydration status

No significant difference was observed in pre-exercise urine osmolality ($t_7 = -1.212$, $p = 0.265$) between TYR (128.8 ± 86.8 mOsm kg^{-1}) and PLA (172.5 ± 102.2 mOsm kg^{-1}). No significant difference was observed in mean sweat loss calculated from pre-post body mass ($t_7 = -0.687$, $p = 0.514$) between TYR (1.6 ± 0.6 L) and PLA (1.7 ± 0.6 L).

Heart rate

A significant effect of time was noted over the 90 min protocol for mean HR ($F_{21,147} = 161.387$, $p < 0.001$) with a mean increase of 97 b min^{-1} and 100 b min^{-1} from 0 min to 90 min in the TYR and PLA conditions, respectively. No significant main effect for condition was observed in mean HR ($F_{1,7} = 4.839$, $p = 0.064$) and there was no significant condition x time interaction effect ($F_{21,147} = 0.88$, $p = 0.62$) (Fig. 1).

Temperature measures

There was a significant effect of time for mean T_{re} ($F_{21,147} = 106.941$, $p < 0.001$), with a significant rise in T_{re} throughout both halves and a decrease back to baseline at HT. End T_{re} at 90 min was 38.7 ± 0.4 °C in TYR and 39 ± 0.2 °C in PLA. No significant main effect for condition was observed in mean T_{re} ($F_{1,7} = 0.65$, $p = 0.447$) between TYR (38.2 ± 0.3 °C) and PLA (38.3 ± 0.2 °C) and no significant condition x time interaction ($F_{21,147} = 1.113$, $p = 0.341$). There was a significant effect of time for mean T_{sk} ($F_{21,147} = 21.679$, $p < 0.001$), with an increase in T_{sk} in both halves and a drop back to baseline at HT. No significant main effect for condition was observed in mean T_{sk} ($F_{1,7} = 0.009$, $p = 0.929$) between TYR (34 ± 0.8 °C) and PLA (34.1 ± 1.4 °C) and there was no significant condition x time interaction ($F_{21,147} = 0.93$, $p = 0.993$) (Fig. 2).

Subjective measures

There was a significant effect of time for TSS ($F_{21,147} = 61.818$, $p < 0.001$) with an increase throughout exercise reaching end values of 6.8 ± 0.4 in TYR and 7.1 ± 0.4 in PLA, indicating that subjects felt 'very hot' at the 90 min stage. No significant main effect for condition was observed in mean TSS scores ($F_{1,7} = 2.154$, $p = 0.186$) between TYR (5.9 ± 0.6) and PLA (6 ± 0.4) and there was no significant condition x time interaction. There was a significant effect of time observed over the 90 min protocol for RPE ($F_{21,147} = 96.536$, $p < 0.001$) with an increase throughout exercise. No significant main effect for condition was observed in mean RPE scores ($F_{1,7} = 2.299$, $p = 0.173$) between the TYR (13.9 ± 1.3) and PLA (14.2 ± 1.3) and no significant condition x time interaction was noted ($F_{21,147} = 0.343$, $p = 0.997$) (Fig. 3).

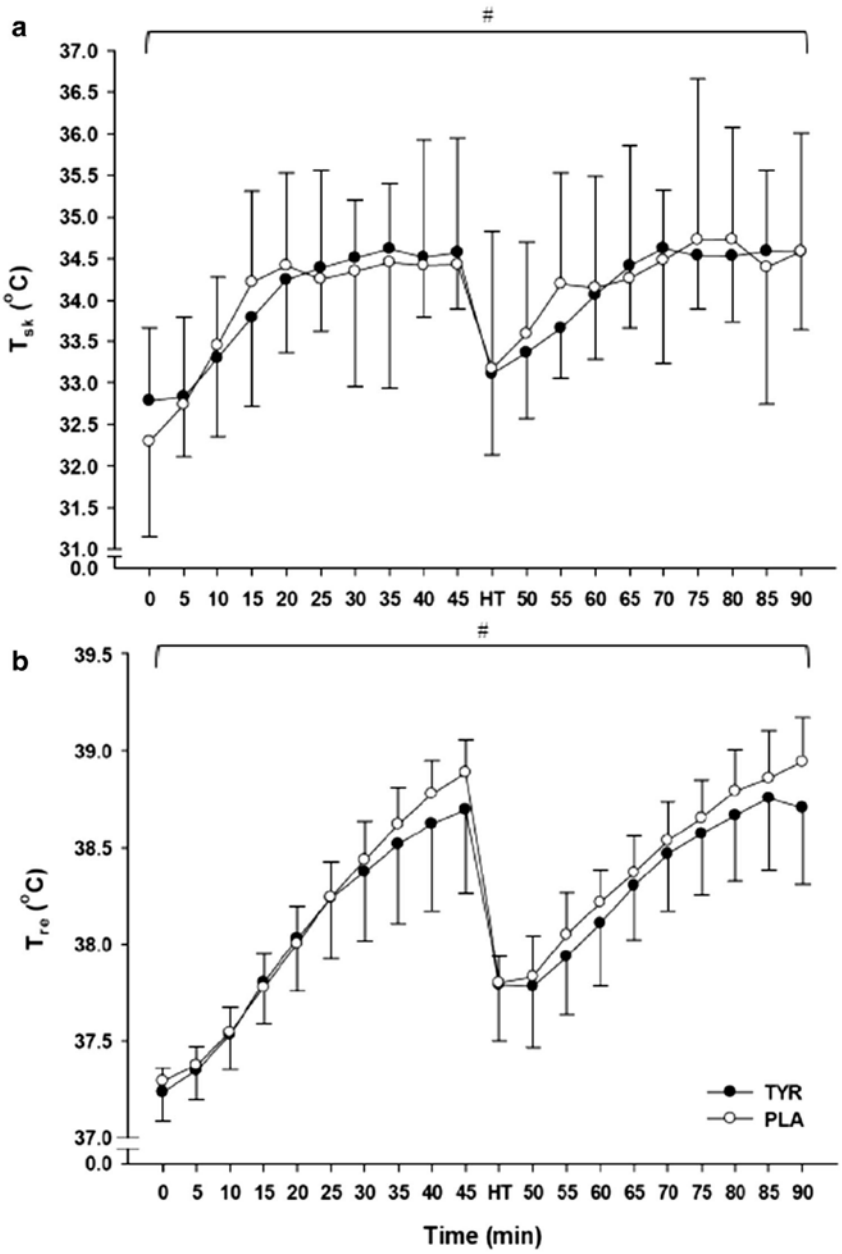

Fig. 2 Group mean-weighted skin temperature (**a**) and mean core temperature (**b**) responses to exercise across both TYR and PLA conditions. Values are mean ± SD. Participants experienced a similar rise in core and skin temperature during both conditions that was not significantly different. #Significant differences over the 90 min protocol ($p < 0.05$)

Effort scales

A significant effect of time was noted for RTIPE ($F_{3,21} = 31.741$, $p < 0.001$) with a decrease at the end of both halves (45 min and 90 min). No significant main effect was observed for subjects RTIPE scores ($F_{1,7} = 0.568$, $p = 0.476$) between the TYR (6 ± 1.7) and PLA (5.6 ± 2.1) conditions and no significant condition *x* time interaction was observed ($F_{3,21} = 2.739$, $p = 0.069$). There was a significant main effect for condition for RTIME scores ($F_{1,7} = 6.443$, $p = 0.039$). On average, RTIME was significantly higher by 13 ± 36 % in the TYR condition compared to PLA ($p = 0.039$, 95 % CI = 6–7). A significant effect of time was noted ($F_{3,21} = 28.745$, $p < 0.001$) with a decrease in RTIME at the end of both halves. However, no condition *x* time interaction was noted ($F_{3,21} = 2.75$, $p = 0.068$) (Fig. 4).

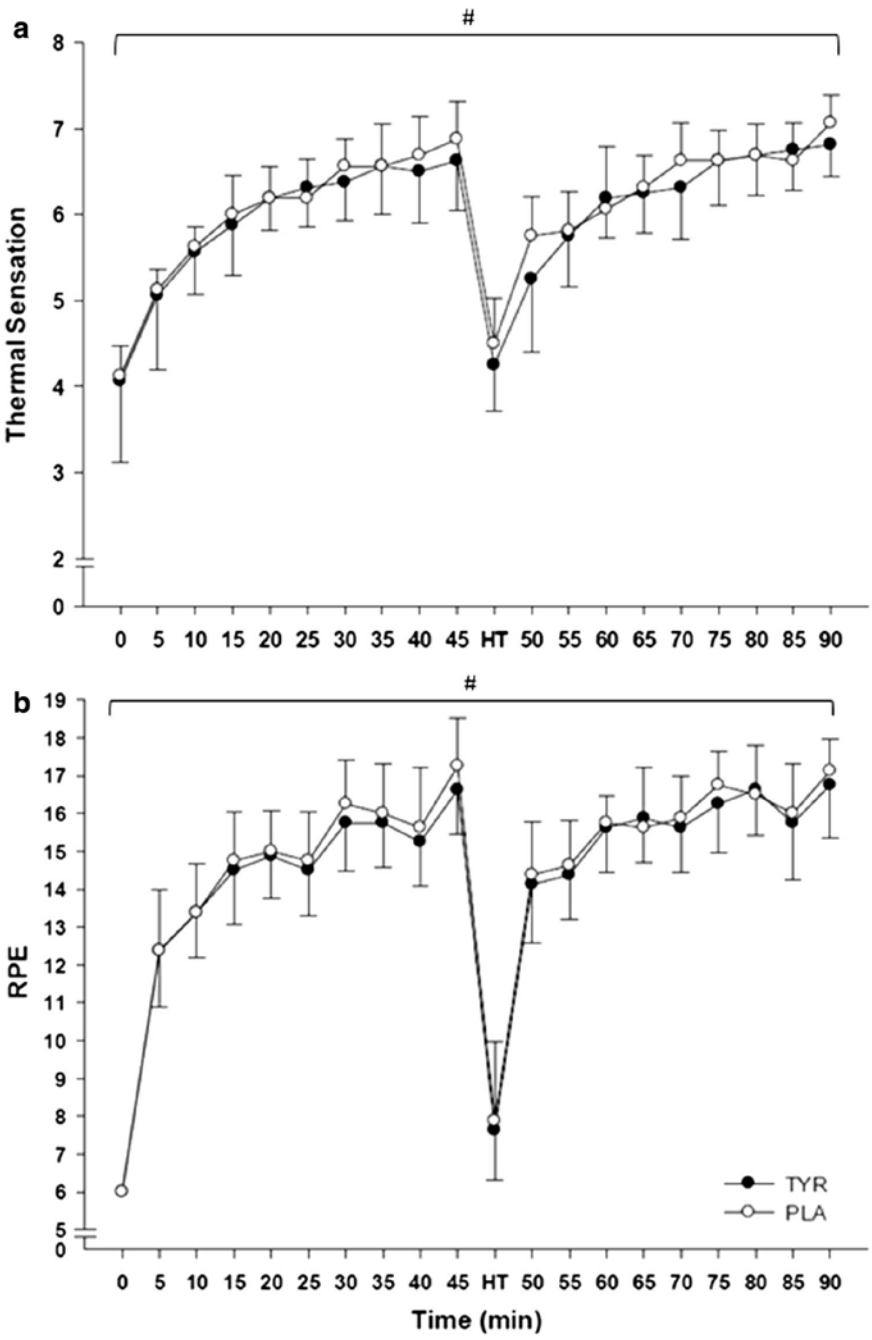

Fig. 3 Group mean thermal sensation (TSS) (**a**) and rating of perceived exertion (RPE) (**b**) responses to exercise across both TYR and PLA conditions. Values are mean ± SD. Participants experienced a similar rise in both TSS and RPE during both conditions that was not significantly different. #Significant differences over the 90 min protocol ($p < 0.05$)

Distance covered

No significant difference was observed in the distance covered in the first half of iSPT ($t_7 = -1.083$, $p = 0.315$) between TYR (4323.6 ± 344.7 m) and PLA (4390.8 ± 241.2 m) or in the second half ($t_7 = -0.747$, $p = 0.497$) between the two conditions (4307.6 ± 378.9 m and 4338 ± 322.7 m, respectively). Overall the total distance covered was not significantly different ($t_7 = -1.025$, $p = 0.339$) between conditions. There was also no significant difference in distance covered between halves in TYR ($t_7 = -0.465$, $p = 0.656$) or PLA ($t_7 = 1.176$, $p = 0.278$) (Fig. 5).

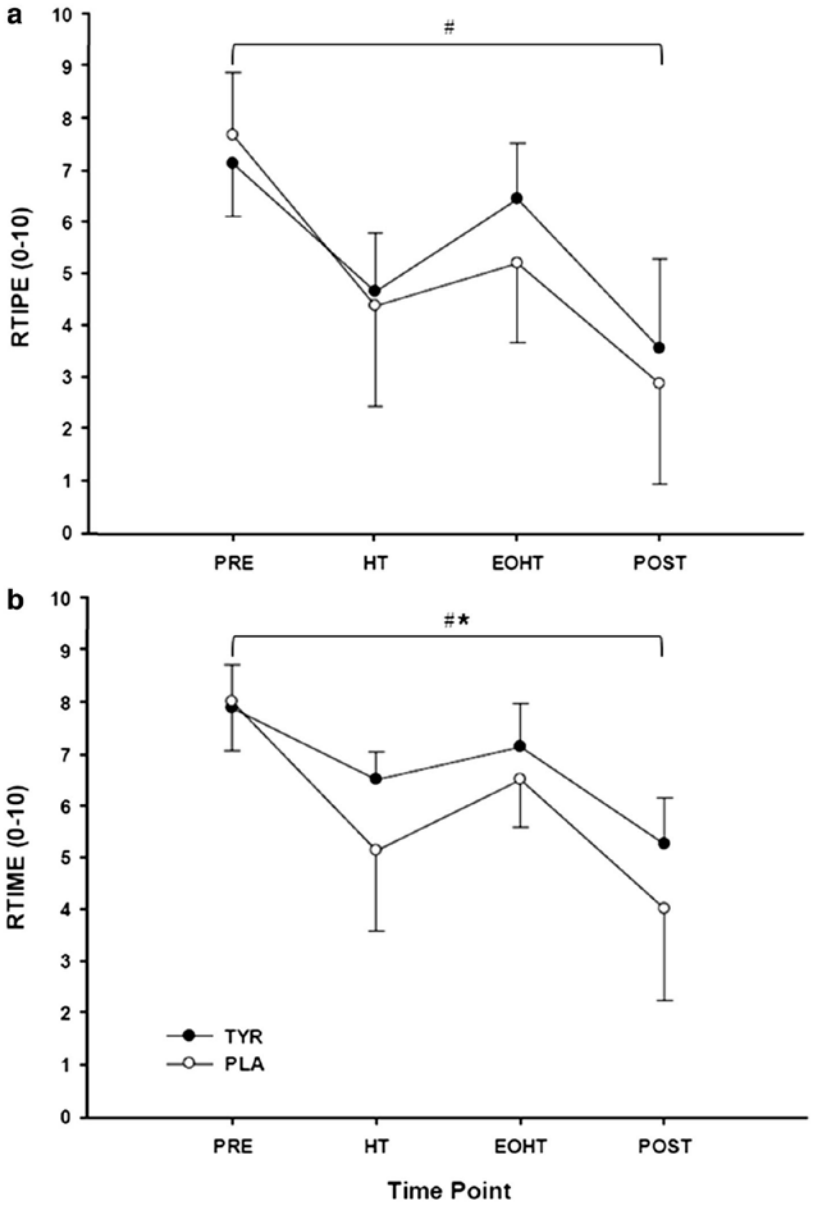

Fig. 4 Group mean readiness to invest physical effort (RTIPE) (**a**) and readiness to invest mental effort (RTIME) (**b**) across both TYR and PLA conditions. Values are mean ± SD. Note: *HT* half-time and *EOHT* end of half-time. *Denotes significant difference between conditions ($p < 0.05$). #Significant differences over time ($p < 0.05$)

Cognitive performance

Vigilance

There was a significant main effect for condition for HIT scores ($F_{1,7} = 10.17$, $p = 0.015$). On average there was a 9 ± 28 % increase in HIT scores in the TYR condition compared to PLA ($p = 0.015$, 95 % CI = 0–2). However, there was no significant condition *x* time interaction ($F_{3,21} = 0.06$, $p = 0.98$) or an effect of time ($F_{3,21} = 0.14$, $p = 0.94$) on HIT scores. There was a significant main effect for condition for MISS scores ($F_{1,7} = 10.95$, $p = 0.013$), with an average decrease of 31 ± 29 % in the TYR condition compared to PLA ($p = 0.013$, 95 % CI = 0–2). However, there was no significant condition × time interaction ($F_{3,21} = 0.05$, $p = 0.83$) or an effect of time ($F_{3,21} = 0.2$, $p = 0.67$) on MISS scores. No significant main effect for condition was observed for

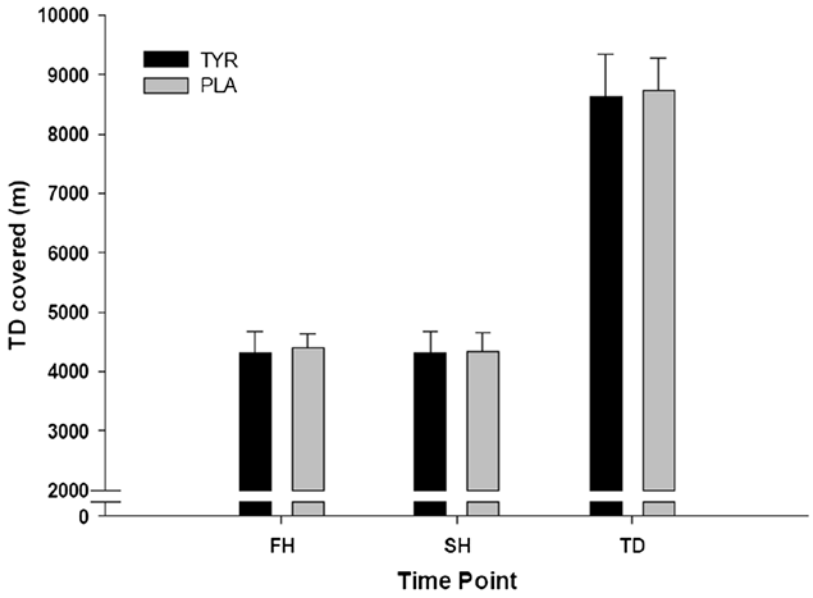

Fig. 5 Group mean distance covered (m) during the first half (FH), second half (SH) and total distance (TD) covered during iSPT across both TYR and PLA conditions. Values are mean ± SD. Participants covered a similar distance during both conditions that was not significantly different

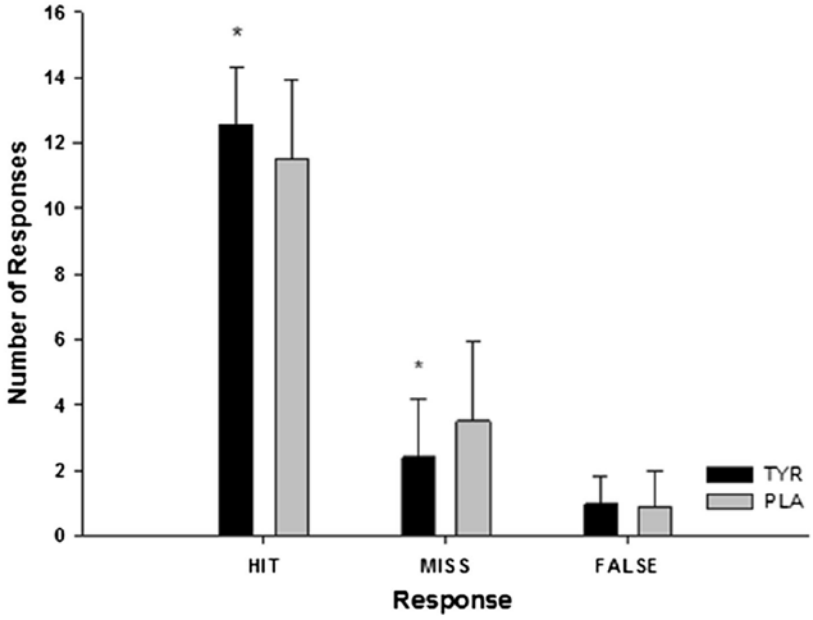

Fig. 6 Group mean vigilance cognitive test responses (HIT, MISS and FALSE) across both TYR and PLA conditions. Values are mean ± SD. *Significant difference between conditions ($p < 0.05$)

FALSE scores ($F_{1,7} = 0.28$, $p = 0.61$) between the TYR and PLA conditions. Furthermore, there was no significant condition x time interaction ($F_{3,21} = 0.77$, $p = 0.52$) or a significant effect of time for FALSE scores ($F_{3,21} = 0.12$, $p = 0.96$) (Fig. 6). Table 2 provides HIT, MISS and FALSE values for each time point (0 min, HT, EOHT and 90 min).

Dual-task

No significant main effect for condition was observed for TRACKING ($F_{1,7} = 1.29$, $p = 0.29$). Furthermore, there was no significant condition × time interaction ($F_{1.65,11.51} = 0.17$, $p = 0.8$) or effect of time ($F_{2.15,15.1} = 1.37$, $p = 0.29$). Similarly, there was no

Table 2 Vigilance scores in TYR and PLA conditions for all time-points measured. Overall main effect observed for HIT and MISS scores in TYR condition

Response	Treatment	Score			
		0 min	HT	EOHT	90 min
HIT	TYR	12.8 ± 2.2	12.6 ± 1.8	12.4 ± 1.4	12.5 ± 1.8
	PLA	11.8 ± 2.1	11.4 ± 2.8	11.5 ± 2.5	11.4 ± 2.8
MISS	TYR	2.1 ± 2.2	2.4 ± 1.8	2.6 ± 1.4	2.5 ± 1.8
	PLA	3.3 ± 2.1	3.8 ± 2.7	3.4 ± 2.6	3.6 ± 2.8
FALSE	TYR	1.2 ± 0.6	0.9 ± 0.6	1 ± 1.3	0.9 ± 0.S
	PLA	0.5 ± 0.8	1.1 ± 0.8	0.8 ± 0.9	1.1 ± 1.7

Values are mean ± SD

Table 3 Dual-task cognitive test scores in TYR and PLA conditions

Response	Treatment	Score
Dual-task TRACKING (%)	TYR	72.3 ± 6.7
	PLA	70.3 ± 6.3
Dual-task MISS	TYR	1.1 ± 0.3
	PLA	1.1 ± 0.5
Dual-task FALSE	TYR	0.3 ± 0.8
	PLA	0.5 ± 0.7

Values are mean ± SD

significant main effect for condition for MISS scores ($F_{1,7} = 0$, $p = 1.0$) and no significant condition × time interaction ($F_{3,21} = 0.17$, $p = 0.8$) or effect of time ($F_{3,21} = 1.37$, $p = 0.28$). Finally, there was no significant main effect for condition for FALSE scores ($F_{1,7} = 0.16$, $p = 0.70$) and no significant condition × time interaction ($F_{1.33,7.95} = 0.49$, $p = 0.56$) or effect of time ($F_{1.57,9.4} = 1.11$, $p = 0.35$) (Table 3).

Discussion

For the first time, the effect of TYR ingestion on soccer-specific exercise (iSPT) and cognitive function within a warm environment (25 °C) was investigated. The main finding of the present study was that a pre-exercise dose of 300 mg kg body mass^{-1} TYR was associated with improved vigilance, accepting the primary hypothesis. Vigilance HIT responses were significantly increased on average by 9 ± 28 % ($p = 0.015$) with MISS responses significantly decreased on average by 31 ± 29 % ($p = 0.013$) in TYR compared to PLA. This improvement was accompanied by a significant increase of 13 ± 36 % ($p = 0.039$) in RTIME in the TYR condition. This novel finding suggests that ingestion of TYR, a catecholamine precursor, may improve cognitive function during exercise-heat stress and possibly influence the perception of psychological effort. However, TYR ingestion had no effect on physical performance, as the distance covered during iSPT was similar in both conditions, which indeed supports the majority of literature in this area (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005; Watson et al. 2012; Tumilty et al. 2014).

The present study provides a novel paradigm for the use of TYR in relation to soccer-specific exercise (iSPT), which offers ecological validity and widens the application of the supplement, from previously military (Banderet and Lieberman 1989; Neri et al. 1995; Deijen et al. 1999; Lieberman et al. 2005; Mahoney et al. 2007) and individual sport (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005; Watson et al. 2012; Tumilty et al. 2014) biased designs. The current findings extend and support the large body of literature demonstrating that TYR is an effective nutritional supplement for alleviating stress-induced deficits in cognitive function (Banderet and Lieberman 1989; Neri et al. 1995; Deijen et al. 1999; Lieberman et al. 2005; Mahoney et al. 2007). During periods of stress, there is a marked decrease in the synthesis of central catecholamine neurotransmitters at the point of exhaustion, inducing partial depletion of catecholamine concentration occurring in the hippocampus and striatum as demonstrated in rodents (Bailey et al. 1993; Meeusen et al. 1997). The proposed mechanism for the provision of supplementary TYR (DA and NA precursor) is to increase central catecholamine neurotransmission, which appears to be advantageous during stressful situations by maintaining facets of cognitive function. It has previously been shown that TYR improves the behavioural response to heat-stress and increases central NA release, albeit in rodents and not humans (Lieberman et al. 2005). The present novel data support this rodent data (Lieberman et al. 2005), with the observed improvement in vigilance in the TYR condition during exposure to exercise-heat stress (Fig. 1). However, mechanistic cause and effect data to support this proposed mechanism are not provided from the employed experimental design, as plasma concentrations of TYR, LNAA or catecholamines were not measured.

In the present study TYR improved subjects vigilance, compared to a placebo, as a main effect (increased HIT and decreased MISS responses, see Table 1 for response descriptions and Fig. 6 and Table 2 for data). This improvement was evident across all cognitive test time points, even

prior to the commencement of iSPT. Thus, on average, TYR supplementation may be beneficial to soccer players throughout match play in warm environments, rather than specifically during the latter stages, when fatigue is suggested to occur (Meeusen et al. 2006a). This finding was coupled with a significant increase in RTIME, implying that subjects felt more psychologically ready after the bouts of exercise-heat stress in TYR. This novel soccer-specific data suggests that TYR may augment mental alertness during periods of stress and as a result, contribute to an increase in cognitive performance. Similar paradigms are seen in military focused research with army personnel reporting 'clearer thinking' and a decrease in adverse moods associated with extreme environmental stress (cold and hypoxia) after TYR supplementation, which coincided with a reduction in cognitive performance impairments (Banderet and Lieberman 1989). Despite the improvement in vigilance, there was no significant difference in the dual-task cognitive test scores between conditions in the present study. Lack of statistical significance within the dual-task test may derive from the high inter-individual variation in performance (e.g. individuals with very good or poor dual-task skills), decreasing the chance of observing statistically significant differences between treatment groups, as identified by Hope et al. (1998).

Despite the sound theoretical basis for the use of TYR, the current study failed to demonstrate any improvement in physical performance after TYR ingestion, showing no change in the distance covered during iSPT between conditions (Fig. 5). Furthermore, no effect of time was observed with subjects covering a similar distance in each half of the protocol, highlighting an absence of fatigue. Fatigue is expected after such high intensity exercise in warm conditions; thus this finding is surprising and may have limited the likelihood of TYR exerting any beneficial effect on physical performance. However, this novel finding, specific to team sport performance (iSPT), is concurrent with several other studies in which no beneficial effect of TYR was observed on endurance performance (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005) in temperate conditions, or exercise to exhaustion (Watson et al. 2012) and a self-paced time-trial (Tumilty et al. 2014) in hot environments. Conversely, two previous studies from the same authors have demonstrated that the availability of TYR influences the capacity to perform exercise in the heat during constant-load cycling (Tumilty et al. 2011, 2013). Tumilty et al. (2011) observed a 15 ± 11 % increase in exercise capacity during a cycling trial in the heat (30 °C). This is the only study to date, to show a physical benefit of TYR ingestion, despite the efforts of Watson et al. (2012) with a comparable exercise protocol, dosage and rise in circulating TYR to Tumilty et al. (2011). Additionally, Tumilty et al. (2013) confirmed that ingesting a TYR/phenylalanine-free amino acid mixture (to deplete blood TYR levels) reduces exercise capacity in the heat compared to a balanced amino acid mixture (containing TYR), which supports the role of TYR availability in exercise-induced fatigue in the heat. However, as the majority of literature (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005; Watson et al. 2012; Tumilty et al. 2014), including the current work, contradicts this recent evidence (Tumilty et al. 2011; Tumilty et al. 2013), it appears that acute ingestion of 150–300 mg kg body mass^{-1} TYR does not provide an ergogenic effect on a plethora of exercise modalities performed in hot, warm and temperate conditions.

It is not completely clear why there are opposing physical performance findings in the aforementioned studies (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005; Tumilty et al. 2011; Watson et al. 2012; Tumilty et al. 2014). One possible reason may be the differences in subject's aerobic fitness, training status and experience with exercise testing between studies as this may influence the effects of TYR on performance; however, this is merely speculation. Furthermore, Tumilty et al. (2014) suggest that the magnitude of activation of the catecholamine system, subsequent to the stress induced by the different exercise protocols, may provide a possible explanation. Under conditions which are not highly stressful, cerebral levels of tyrosine hydroxylase are saturated with substrate; thus the use of supplementary TYR should not significantly increase central catecholamine synthesis or improve exercise tolerance or performance (Lehnert et al. 1984; Foley and Fleshner 2008). Hence it is not surprising that previous studies investigating the effects of TYR in temperate conditions (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005) failed to observe an ergogenic effect. By utilising the iSPT protocol in the present study, we attempted to combine intense physical exertion with elevated ambient temperature (25 °C) to create a sufficiently demanding environment to alter central catecholamine neurotransmission. However, the protocol did not produce a sufficiently stressful environment as anticipated, recording very similar end HR values (175 and 177 b min^{-1} in TYR and PLA, respectively) to Watson et al. (2012) (177 and 175 b min^{-1} in TYR and PLA, respectively) and Tumilty et al. (2011) (174 and 177 b min^{-1} in TYR and PLA, respectively). As iSPT is an individualised, valid and reliable protocol with regard to internal and external load of soccer, increasing the intensity per se of the protocol is precluded for such reasons. However, increasing the ambient temperature (from 25 to >30 °C) that iSPT is performed within could likely manifest a sufficient level of stress to up-regulate catecholamine turnover. Increasing the environmental temperature may also induce higher T_{re} values, contributing to the attainment of a 'critical' internal temperature (38 > 40 °C) (Cheung and Sleivert 2004), which is suggested to coincide

with exhaustion during prolonged exercise in the heat (Nielsen et al. 1993; González-Alonso et al. 1999).

The methodology of the present study contains several limitations, which should be considered in future research. As previously mentioned, the present study did not assess plasma concentration/ratio of TYR and LNAA, which limits cause and effect relationships. However, as previous studies observed significant elevations in plasma/serum TYR after administering 150 mg kg body mass^{-1} (Tumilty et al. 2011; Watson et al. 2012), it is assumed that a similar, if not greater rise may have occurred in the present study after ingestion of a double-dose (300 mg kg body mass^{-1} TYR in total). An investigation into the pharmacokinetics of TYR levels in the blood, using a variety of doses, would perhaps provide further elucidation and a basis for future exercise studies. Moreover, the TYR supplement administered in the present study was sourced from an online sport nutrition company, the same company used by Tumilty et al. (2011). This issue was highlighted by Watson et al. (2012) due to the known uncertainty relating to the composition of some widely available nutritional supplements. Although this is important to consider, the TYR supplement used in the present study was analysed via HPLC to assess its purity, which was found to be satisfactory (>90 %). However, we highly recommend that future research utilise supplements from medical nutrition companies to minimise risk of contamination, in line with Watson et al. (2012). Furthermore, the timing of the cognitive assessments (PRE, HT, EOHT and POST) may also be considered a limitation. There is evidence to suggest that cognitive function may be impaired or disturbed during maximal exercise (McMorris and Keen 1994), with a rapid return to baseline after cessation of exercise (Dietrich and Sparling 2004). Such disturbances may be due to a larger cerebral emphasis on motor outputs during exercise, at the expense of the cognitive tasks (Dietrich and Sparling 2004). Therefore, future work should aim to evaluate cognitive function during the employed exercise protocol. Finally, the cognitive assessments were all completed post-ingestion of the supplement, which does not allow for a pre-post ingestion comparison to be made. Although this may have improved the study design, the additional repetition of the cognitive tests may have become tedious and consequently decrease the engagement of the subjects.

As soccer is highly dependent upon the execution of motor skills and decisional-based tasks, minor decrements in cognitive performance could significantly alter the outcome of a game (Meeusen et al. 2006a). Since many important soccer matches and tournaments are played in hot climates (>25 °C), including the Champions League and World Cup finals, ingestion of TYR as a pre-game supplement may enhance the decision-making capabilities of soccer players. Additionally, on-pitch referees may also benefit from TYR supplementation, as previous research has shown that elite referees cover a similar distance to players during a game (Weston et al. 2011); thus similar internal and external loads are experienced by referees [iSPT replicates these loads in an individualised, valid and reliable manner (Aldous et al. 2014)]. The use of the newly validated iSPT (Aldous et al. 2014) protocol to replicate individualised internal and external soccer-specific loads provides novel data regarding TYR supplementation within team sport based exercise. Furthermore, as previous military research exclusively explores cognition and mood state with and without TYR supplementation within cold and/or hypoxic conditions (Banderet and Lieberman 1989; Mahoney et al. 2007; O'Brien et al. 2007), the present findings may provide a stimulus for exploration within hot environments, as army personnel may undergo similar exercise-heat-stress situations during training and deployment overseas. The current study replicated the maximum dose (300 mg kg body mass^{-1} TYR) previously administered in the literature (Mahoney et al. 2007; O'Brien et al. 2007) in a drink form, without any adverse side effects, which may be useful for future research investigating large doses of TYR.

Conclusions

In summary, this study demonstrated for the first time that ingestion of 300 mg kg body mass^{-1} TYR significantly improved vigilance and RTIME, but not physical performance, when exposed to individualised soccer-specific exercise (iSPT) in a warm environment. This suggests that TYR availability is associated with improvements in aspects of cognitive performance when exposed to acute stress and, therefore, may be beneficial as a nutritional supplement prior to soccer match-play in hot conditions. The exact mechanism to explain these findings is at present unclear and although previous literature (Lehnert et al. 1984; Lieberman et al. 2005; O'Brien et al. 2007) may provide reasonable speculation, these concepts must be explored further before definite conclusions can be made. Future research should investigate the pharmacokinetics of TYR and also assess the effects of chronic supplementation on health and performance.

Conflict of interest None.

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Appendix H

Screenshot of MSSE publication related to this thesis – full text PDF is currently unavailable due to proofing process

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Tyrosine Ingestion and Its Effects on Cognitive and Physical Performance in the Heat.

Coull, Nicole; Chrismas, Bryna; Watson, Phillip; Horsfall, Rachel; Taylor, Lee

PAP

Abstract

PURPOSE: Ingestion of tyrosine (TYR), a catecholamine precursor, has previously improved aspects of cognitive function and mood during acute stress, though there is limited research exploring the optimal dose relative to blood values. The serum response of two doses of TYR were investigated (study 1) with the identified 'optimal' dose assessed relative to cognitive and physical performance during a military-based protocol in the heat (study 2).

METHODS: Study 1: Twenty-one participants were assigned to one of three groups; HIGH (2 doses of 150 mg.kg body mass-1 TYR), LOW (2 doses of 75 mg.kg body mass-1 TYR) and CON (sugar-free drink). Participants ingested TYR in two separate doses (0900 and 1300) and remained in the laboratory from 0800-1700 having blood drawn every hour. Study 2: Eight participants completed a military-based load carriage protocol comprising a 60 min walk (6.5 km.h), followed by a 2.4 km time-trial carrying a 25kg backpack (40[degrees]C; 30% RH) on two occasions (TYR/placebo), in a double-blind counterbalanced crossover design. Cognitive function was assessed before, during and after exercise.

RESULTS: Study 1 demonstrated that ingestion of a single dose of 150 mg.kg body mass-1 TYR was equally as efficient at elevating serum TYR concentration relative to a double dose. In study 2, exercise-heat-stress impaired some aspects of cognitive function; however TYR did not alleviate these decrements ($p > 0.05$). Furthermore, no difference was observed in any physiological variable between conditions ($p > 0.05$) or time-trial completion time ($p = 0.74$) between TYR (19.78 +/- 3.44 min) and PLA (20.29 +/- 3.55 min).

CONCLUSION: Despite marked elevations in serum TYR concentration, ingestion of TYR did not influence cognitive function or physical performance during exercise-heat-stress.

Appendix I

Power calculation for experimental chapter 1